SERIAL ANALYSIS OF rRNAS (SARS): A NOVEL MOLECULAR STRATEGY FOR SAMPLING MICROBIAL DIVERSITY THROUGHOUT THE UNIVERSE.

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ABSTRACT

The most effective means to expand our narrowed view of Earth's microbial diversity is the use of molecular techniques that target ribosomal RNA and other well conserved genes. Molecular data can provide evidence of organisms in an environment without requiring their isolation and growth in the laboratory. However, exhaustive inventories of microbial communities are still impractical. Serial Analysis of rRNAs (SARS) is a new high-throughput technique that can sample at the sequence level short species-specific regions of PCR amplified rRNAs genes from many microorganisms in a single reaction. This provides a means to rapidly and efficiently enumerate relative numbers of rDNA templates in a nucleic acid population extracted from a microbial community. In this communication we describe the application of this technique to an extreme environment, the hydrothermally active sediments of the Guaymas Basin (Gulf of California, Mexico).

1. STATEMENT OF THE PROBLEM

Recent observations of extra-solar system planets and the discovery of microbial life in extreme environments, heightens our expectations that life will likely be encountered beyond Earth. A better understanding of the microbial ecology of extreme environments on Earth may 1) enlighten us about the origins and full diversity of life, 2) provide insights into the limits of the adaptability of life, and 3) redefine the environmental boundaries that are compatible with the search for life on other planets.

Historically, culture dependent methods and morphological identification of microorganisms have been the only way to unambiguously identify a distinct microbial species. However, with the availability of molecular genetic techniques, scientists began to develop new ways of identifying microorganisms. Molecular biology methods have provided a most effective means to expand our narrowed view of microbial diversity on Earth and have lead to the development of new ideas about the origin and evolution of life [1]. Ideally large data sets should be gathered to provide reliable estimates of total microbial diversity, but exhaustive inventories are not yet possible. The inherent expense and limited throughput of individual sequencing reactions has led to the widespread use of terminal restriction fragment length polymorphisms (T-RFLP, [2]) or denaturing gradient gel electrophoresis (DGGE, [3]) to characterize products of polymerase chain reaction (PCR) experiments. But these techniques provide very low levels of information about microbial diversity and the results are difficult or impossible to compare between different sites. Therefore, a question arises: Is there a way to obtain quantitative estimates of natural diversity in an exhaustive manner?

2. SERIAL ANALYSIS OF RIBOSOMAL RNAS (SARS)

Serial Analysis of Ribosomal RNAS (SARS) is a new high-throughput technique that promises to revolutionize studies of microbial population structures in both nominal and extreme environments.

SARS is based on sequence analyses of ribosomal RNAs (rRNAs) as indicators of microbial diversity and population structure. The technique provides a means to rapidly and efficiently enumerate relative numbers of ribosomal DNA templates in a nucleic acid population extracted from a natural environmental sample.

SARS technology is modeled after serial analysis of gene expression (SAGE, [4]), which describes relative gene expression patterns. In SARS, the PCR products from a selected, usually rapidly evolving (hypervariable) region in rRNA genes, are ligated together to form large concatemers (Fig.1). A single DNA sequencing reaction of a cloned concatemer can describe many of these chosen regions represented in a population of nucleic acid molecules. In this way, samples loaded onto a 96 channel capilary sequencing machine can provide information about thousands of microorganisms in an analyzed sample (depending on clone length and sequencing processivity). It is possible to design primers to target hypervariable regions of rRNA specific to any of the three primary domains or specific to phylogenetic clades. Moreover,

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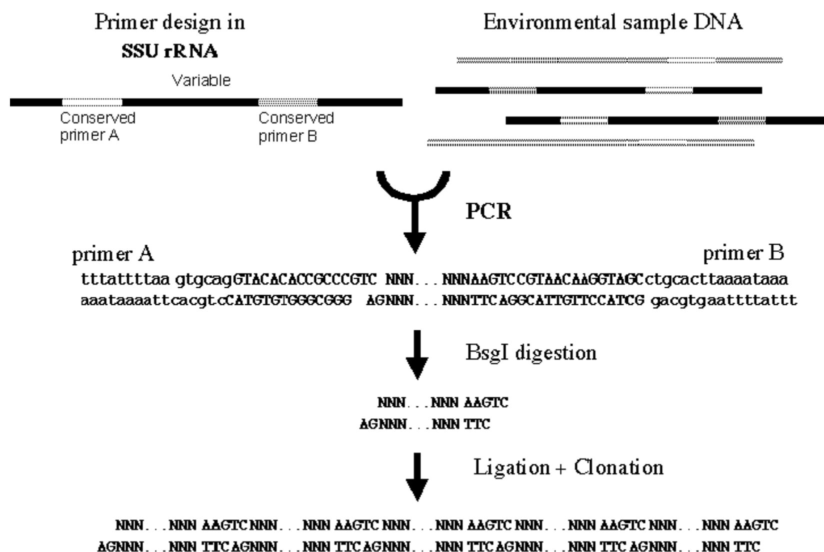

Fig 1. General methodology of the SARS technique. The SARS technology sample using conserved primers that flank a hyper variable region (20-100bp) from the small subunit of ribosomal RNA (SSU rRNA) and contain a type IIs restriction site (BsgI). The primer sequences are removed from the amplicons by restriction digestion with BsgI leaving a short base extension. A biotin-streptavidine cleaning step allows to physically separate digested fragments from biotinylated primers, which remain attached to the streptavidin beads. Eventually, digested fragments are ligated to form concatemers that are then cloned and sequenced

the simplicity of the technique allows broader applications. With appropriate primer design, we can target slowly evolving rRNA genes to obtain information about diversity at higher taxonomic levels or, alternatively, we can target protein coding genes that have different evolutionary "clock speeds" or specific functions relevant to a particular environmental niche.

3. PRELIMINARY RESULTS TO VALIDATE SARS

Funded through the NASA Astrobiology Institute, molecular-based studies of hydrothermal active sediments of the Guaymas Basin (Gulf of California, Mexico) have reported microbial diversity inventories for several core samples [5]. We have applied SARS to the same biological samples. Using bacterial universal primers that amplify a hypervariable region of prokaryotes 16S rRNA gene we obtained more than 500 sequences in samples of core A1 of the Guaymas Basin. The information content of these sequences eclipses our previous studies based upon partial rRNA sequence comparisons. A preliminary analysis of these sequences indicates that major representatives are Beggiatoa, known to be an abundant organism in this vent, as well as a deeply branching phylotype so far only found in core A of the Guaymas Basin, and *Thermodesulfobacterium*, a genus marginally affiliated to this deeply branching phylotype [5]. Three of the most common bacterial phylotypes previously found in core A i.e. delta and epsilon Proteobacteria and Green non-sulfur bacteria were also well represented in the SARS analysis. Also in agreement with previously reported we recovered other sequences affiliated to other bacterial lineages in smaller numbers including Planctomycetales, Verrucomicrobium, and uncultured OP3 and OP1. In contrast to our previous study, we retrieved several sequences affiliated to gamma and alpha Proteobacteria not found to date in this core.

CONCLUSIONS

Although preliminary, our comparative study supports predictions on how powerful SARS methodology can be to detect microbial population structures in natural samples. Clearly a broad application of SARS technology will accelerate Astrobiology studies that seek to define environmental boundaries that are compatible with the search for life on other planets, as well as the conditions conductive to life's origins and evolution.

4. REFERENCES

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