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Thermophilic Bacteria: Their Relationships to Other Bacteria and Role in Archaebacterial and Eubacterial Evolution

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19 ABSTRACT (Continue on reverse if necessary and identify by block number)

The purpose of the continuing research is to study the microbial life of thermophilic habitats in terms of microbial diversity and phylogenetic relationships. Ribosomal RNA sequences are generated and examined in a comparative framework to determine the phylogenetic distances of several new thermophilic (anaerobic) bacterial isolates.

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PROGRESS REPORT, CONTRACT NOO14-86-K-0278

PRINCIPAL INVESTIGATOR: C. R. Woese

TITLE: THERMOPHILIC BACTERIA: THEIR RELATIONSHIPS TO OTHER BACTERIA AND ROLE IN ARCHAEBACTERIAL AND EUBACTERIAL EVOLUTION

1. Project Goals

The main goal of the present study is to examine what effect the thermophilic condition had on the early evolution of the cell and, specifically, to identify any possible molecular constraints this condition placed on the ribosomal RNA.

2. Progress and Plans for Third Year

a. Progress

During the past year we have characterized the 16S ribosomal RNAs from several novel thermophilic isolates provided by Prof. Karl Stetter in addition to isolates from various other sources. The rRNA has been directly sequenced by the dideoxy termination method using reverse transcriptase and primers designed specifically for the 16S rRNA molecule.

The thermophiles examined have been isolated from a wide variety of thermophilic niches in sources geographically removed from one another (e.g. the Red Sea, Indonesia, Azores, deep sea vents). Although isolated from varied and distinct thermophilic habitats, the new bacterial strains are generally representative of previously defined phylogenetic groups. This agrees with our earlier observation that there appears to be relatively limited phylogenetic diversity in thermophilic niches, as determined from the organisms thus far isolated from these environments. Keywords: Archaebacteria; eubacteria; thermophilic bacteria; (KT)

One extreme thermophile studied, "*Pyrococcus*," which grows at temperatures exceeding 100° C, was found to be a close relative of the previously characterized species *Thermococcus celer*.

Another thermophile under investigation, *Acidianus infernus*, has the capability of utilizing sulfur by two different metabolic pathways: anaerobically by the reduction of sulfur compounds or aerobically by the oxidation of elemental sulfur. The rRNA sequence data identifies this organism as a member of the genus *Sulfolobus*.

Several of the new isolates group phylogenetically with the previously characterized eubacterium *Thermotoga maritima*, further defining this deep branching genus. Two of the thermophiles examined, YSRA and KC4, are also deep branching eubacteria although there is no specific relationship to *T. maritima*. Thus, the deepest branching representatives of the eubacterial kingdom (yet known) are, without exception, thermophilic, which is also the

case for the archaebacterial kingdom.

A bacterium isolated from the Red Sea by Prof. Karl Stetter has proven to be difficult to grow in the quantities required for RNA sequencing. Initial work, however, has characterized this organism as a eubacterium.

An important observation emerging from this research is that the rRNAs of thermophilic species tend to be more closely related to one another than expected. To further analyze this "convergence" effect, thermophilic/mesophilic pairs of archaebacteria were examined (e.g. *Methanococcus vannielii* and *Methanococcus jannaschii*). The thermophilic member of the pair is closer in sequence to a given eubacterium than its mesophilic partner in almost every case. Thermophilic convergence, therefore, appears to reflect the fact that the form upon which the thermophilic rRNAs converge somehow resembles the universal ancestral form of rRNA.

b. Plans for Forthcoming Year

The forthcoming year's research strategy involves a continuation of the work on novel thermophilic bacteria to include isolates from the Guaymas Basin, Gulf of California, by Prof. Karl Stetter. We hope to obtain the broadest representation of thermophiles possible from this and other sources. Further phylogenetic studies on the Red Sea isolate (as discussed above) will also continue.

In addition to the phylogenetic placement of new isolates, the existing database will possibly be extended to include more thermophilic/mesophilic pairs for convergence study. Through these and other analyses, emphasis will be placed on analyzing the thermophilic convergence effect in an effort to gain an understanding of the thermophilic nature of the universal ancestor.

3. Publications

Achenbach-Richter, L., K.O. Stetter, and C.R. Woese. 1987. A possible biochemical missing link among the archaebacteria. Nature (London) **327**:348-349.

Achenbach-Richter, L., R. Gupta, K.O. Stetter, and C.R. Woese. 1987. Were the original eubacteria thermophiles? System. Appl. Microbiol. **9**:34-39.

4. Graduate students supported

Laurie Achenbach-Richter