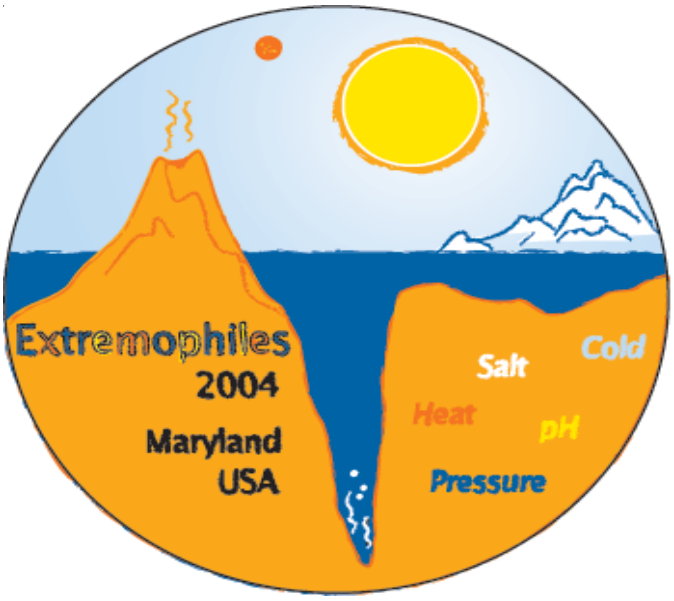

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ISOLATION AND CULTURE OF CARBON MONOXIDE DEPENDENT THERMOPHILES AND HIGH TEMPERATURE PHOTOSYNTHETIC MATS FROM THE UZON CALDERA, KAMCHATKA

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The geothermal sites of the Uzon Caldera, Kamchatka, provide unique opportunities to determine the relationship between microbial and geochemical transformations during mineral formation. In this study, two sites within the Uzon Caldera have been characterized, using microbial culturing and detection techniques. A bacterial mat from the K4 well area in the Central Thermal Field of the caldera was transported to US laboratories, and successful cultures of the mat were maintained at 45, 50 and 55^{0}C. The bacterial populations of the mats were analyzed using denaturing gradient gel electrophoresis (DGGE), culture and isolation of bacterial strains, and cloning and sequencing of 16SrRNA from isolates and the mat populations. In addition, hot spring sediment and the mat cultures were subcultured into anaerobic media that contained CO as the sole carbon and energy source. Isolates obtained were anaerobic, CO-oxidizing thermophiles that clearly survived transportation of the mat at room temperature from Russia under aerobic conditions, and were then established in consortial growth of the mats. DGGE revealed that the isolates were represented in the mat bacterial population, and DNA samples extracted from original mat material in the field. The cultured mat was less diverse than the original mat sample, however many shared bands were observed. Sequencing of full length 16S rDNA clones revealed that the mat population contained several novel bacterial species, and was dominated by Chlorogloeopsis sp. (51% of the sequenced clones). The presence of novel carboxydotrophic bacteria will allow us to address the functions of strictly anaerobic, hydrogenogenic bacteria in these mats that are energized by oxygenic photosynthesis.

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COMPARISON OF 16S RRNA GENE SEQUENCES OF THE GENUS *METHANOBREVIBACTER*

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The phylogeny of the genus *Methanobrevibacter* was established almost 25 years ago on the basis of the similarities of the 16S rRNA oligonucleotide catalogs. Since then, many 16S rRNA gene sequences of newly isolated strains or clones representing the genus *Methanobrevibacter* have been deposited in the public databases. In addition to 16S rRNA gene sequence similarity (*S*), genomic DNA reassociation (*D*) values are also essential for the correct identification of a strain. The statistical implications of the correlation between these two parameters are of great interest in prokaryotic systematics and it depends on the taxa in study. We therefore revised the taxonomic affiliation of the isolates and clones of the genus *Methanobrevibacter,* based on 16S rRNA gene sequences, deduced group specific nucleotide positions showing specific nucleotide substitutions in the 16S rRNA gene sequences, and studied the correlation between *D* and *S* based on the available DNA hybridization data. Our analysis based on 786 bp aligned region from 54 representative sequences of the 120 sequences available for the genus revealed seven multi-member groups namely, Ruminantium, Smithii, Woesei, Curvatus, Arboriphilicus, Filiformis, and the Termite gut symbionts along with three separate lineages represented by *Mbr. wolinii, Mbr. acididurans,* and termite gut flagellate symbiont LHD12. The cophenetic correlation coefficient (0.913), a test for the ultrametric properties of the 16S rRNA gene sequences used for the analysis, indicated the high degree of goodness of fit of the tree topology. A significant relationship was found between *S* and *D* with the correlation coefficient (*r*) for log*D* and log*S*, and for [ln(-ln*D*) and ln(-ln*S*)] being 0.73 and 0.796 respectively. Our analysis suggested that D would be less than 70 % at least 99 % of the times when *S*= 0.984, and with 70% *D* as the species "cutoff", any 16S rRNA gene sequence showing <98% sequence similarity can be considered as a separate species for this genus. All the 16S rRNA gene sequences used in the analysis were supported by the possession of a signature sequence (5'-tgt gag (a/c)aa tcg cg-3', corresponding to *E. coli* positions 375-388) and a nucleotide bulge (5'-T_n-3', n = 6 or 8; corresponding to a stem-loop structure at *E. coli* positions 200-218) except *Mbr. curvatus* (which instead possess the sequence 5'-ttc tta tgt t-3'). We propose to include the termite gut flagellate symbiont LHD12, the methanogenic endosymbionts of the ciliate *Nyctotherus ovalis,* and rat feces isolate RT reported earlier, as separate species of the genus *Methanobrevibacter*. For organisms that have never been isolated but have been detected in natural samples by rRNA sequence alone (like the majority of sequences in the present study), the ability to estimate *D* will provide a clearer understanding of their genetic and phenotypic diversity.