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Microbial community analysis in hydrothermal environment by genomic technology

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It was indicated by microscopic observation or comparison of 16S rDNA sequence that many extremophiles were surviving in many hydrothermal environments. But it is generally said that over 99 % of total microbes are now uncultivable. Thus, we planned to identify uncultivable microbes through direct sequencing of environmental DNA. At first, shotgun plasmid libraries were directly constructed with the DNA molecules prepared from mixed microbes collected from low-temperature hydrothermal water at RM24 in the Southern East Pacific Rise (S-EPR). It was shown that the sequences of some number of clones indicated the similar feature to the intron in eukaryote or tandem repetitive sequence identified in some human familiar diseases. The results indicated that many microorganisms with eukaryotic feature were dominant in low temperature water of S-EPR. Secondly, shotgun plasmid libraries were constructed from the environmental DNA prepared from Beppu hot springs. The ORFs were easily identified all clones determined entire sequence. Thus it can be said that hot springs is good resources for searching novel genes. At last, the mixed microbes isolated from Suiyo seamount were used for construction of shotgun library. The clones in this library contained the ORFs. From some clones in hot spring and Suiyo sample, aminoacyl-tRNA synthatase, which is generally present in all organisms, was isolated by similarity. The phylogenetic analysis of aminoacyl-tRNA synthetase identified indicated that novel and unidentified microorganisms should be present in hot spring or Suiyo seamount. Our work indicates that environmental genomics, direct cloning and sequencing of environmental DNA, is powerful approach to collect novel uncultivable microbes or novel genes.

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Thermodynamic analysis of microbial energy production and consumption in the deep biosphere

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All known forms of life take advantage of oxidation-reduction reactions to convert a wide variety of chemical compounds into *ATP* and/or other generic forms of energy such as electrons and protons that are metabolically useful. However, the quantitative thermodynamic relationships among these redox reactions and endergonic cellular functions such as protein synthesis are not well understood, especially at the high temperatures and pressures encountered in parts of the deep biosphere. Quantifying each step in the energy conversion process would require thermodynamic properties of a plethora of biomolecules such as lipids, membrane-bound proteins, enzymes, limiting nutrients, and other intermediate compounds. Nevertheless, the overall process can be quantified by first establishing a series of interconnected stationary states for which chemical affinities ($\boldsymbol{A}$) can be calculated from $\boldsymbol{A} = 2.303RT\log(K/Q)$, where Q refers to the activity quotient for the reaction. For example, overall microbial energy generation can be described in terms of a series of three stationary states consisting of subcellular units separated by thin membranes, each of which constitutes a selectively open system into and out of which certain reactants and products diffuse at equivalent rates. In the case of heterotrophic sulfate-reducing hyperthermophilic microbes in the deep biosphere, one such set of three stationary states corresponds to

$$C_2H_4O_2 + 0.5SO_4^{2-} + 2NAD_{ox}^{-} \rightarrow H^+ + 0.5H_2S + 2CO_2 + 2NAD_{red}^{2-} \quad (1)$$

$$2NAD_{red}^{2-} + 2MgADP^{-} + 2HPO_4^{2-} \rightarrow 2NAD_{ox}^{-} + 2MgATP^{2-} + 2H_2O + 4e^{-} \quad (2)$$

$$2MgATP^{2-} + 4AA_i \rightarrow 2MgADP^{-} + 2HPO_4^{2-} + UP + 2H^+ \quad (3)$$

where $C_2H_4O_2$ corresponds to acetic acid, NAD_{ox}^{-} and NAD_{red}^{2-} refer to the oxidized and reduced forms of nicotinamide-adenine dinucleotide, $MgADP^{-}$ and $MgATP^{2-}$ denote the magnesium-complexed adenosine di- and triphosphate species, and AA_i and UP stand for the ith amino acid and an unfolded protein, respectively. Using representative activities reported in the literature for reactants and products in deep ecosystems at 100°C, the chemical affinities of each of the above stationary states are ≥20 kcal mol^{-1}, which fuels the *ATP* cycle. Note that the hydrolysis of *ATP* depicted in Eqn. (3) can instead be coupled to other polymerization reactions such as the synthesis of *RNA* and *DNA* in the deep biosphere.