

Microbial Resources in Marine Environments

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Abstract

Studies on marine microbial diversity using direct analysis of rRNA sequences have revealed previously unrecognized microbes and novel phylogenetic lineages that represent major components of global microbial assemblages. This diversity in the marine biosphere offers opportunities for research and application in the field of biotechnology; global gas exchange, nutrient and element cycling, biomass and food production, marine bioproducts, and bioprocesses. Especially, deep-sea encompasses the extremes of virtually all environmental parameters found on Earth and provides extreme microorganisms. In this study several extreme microorganisms were successfully isolated from the deep-sea sediment samples obtained by joining ocean cruises for last 2 years and some of them will be introduced.

Introduction

The oceans constitute more than 70% of the earth's surface, of which about 60% is covered by water more than 2,000 m deep. Among the total number of prokaryotes estimated to be $415-640 \times 10^{28}$, most of the earth's prokaryotes occur in ocean and oceanic subsurfaces, where the numbers of cells are 1.2×10^{29} , 3.5×10^{30} , respectively (Whitmann, 1998). Studies of the ocean environments have led to significant new discoveries of unusual biodiversity, metabolic activity, and natural products of interest to biotechnology. Marine microbial diversity is extremely high, which is a direct consequence of the extraordinary variability of the marine biosphere. This biosphere encompasses a huge thermal range (-1.5°C in Antarctic waters to temperatures in excess of 100°C in shallow and 350°C in deep hydrothermal vent), pressures ranging from 1 to over 1,000 atmosphere, nutrient variations ranging from eutrophic to oligotrophic, and extensive photic and aphotic zones. Most of the metabolic and biochemical diversity of life resides in microorganisms including the domains *Bacteria* and *Archaea*, and unicellular members of the *Eukarya*. There are much potential of marine microbial diversity for commercial development and exploitation in every area of biotechnology (Deming, 1998). In this study we have obtained diverse marine biological samples and isolated microorganisms for the biotechnological utilization of the marine bioresources.

Psychrophiles

Psychrophilic bacteria were isolated from deep-sea sediments of Kuril Trench (6,230 m), East Sea

(3,200 m), and Ayu Trough (4,000 m). Amplified ribosomal DNA restriction analysis (ARDRA) of 51 isolates has shown 16 different RFLP types of which representative isolates were further classified based on 16S rDNA sequencing. Bacterial isolates belong to four phylogenetic groups, alpha and gamma subdivision of the *Proteobacteria*, gram-positive bacteria, and *Cytophaga-Flexibacter-Bacteriodes* (CFB) group. The 16S rDNA sequence similarity of the isolates was ranged from 92.4 to 99.7% to the sequences of the nearest neighbors in database. Nine strains (56%) among isolates were affiliated into gamma-*Proteobacteria* subdivision. A strain, LT17 belongs to the *Shewanella* species known as one of piezophilic psychrophiles based on 16S rDNA sequence. Growth occurs between 10 and 20°C and optimal growth temperature is 10 to 15°C. This strain is rod-shaped (length; 1.0 ~ 1.5 μm, width; 0.5 ~ 0.8 μm). The fatty acid profile of the LT17 was similar to those of other *Shewanella* species. The main cellular fatty acid consists of monounsaturated acid, mainly 16:1ω7. The omega-3 polyunsaturated fatty acid, eicosapentaenoic acid (20:5ω3, EPA) was detected at 11% of the total fatty acids.

An anaerobic and psychrophilic microbial consortium was isolated from Edison seamount sediments (1,500 m) using ZoBell broth containing sulfur under anoxic condition at 4°C. Analysis of sequences of several 16S rDNA clones has shown that those clones belonged to members of genus *Fusobacteria* with high similarity to *Clostridium rectum*, *Fusobacteria* bacterium Ko711, *Propionigenium* sp. MGP-13AN and uncultured *Fusobacteria* bacterium.

Thermophiles and hyperthermohiles

Thermophilic bacteria were isolated from deep-sea sediments of Ayu Trough (4,000 m depth) in the western Pacific area. Among the 21 thermophilic isolates which were enriched at 55°C, 10 representative bacterial strains showing different RFLP type were chosen for the molecular classification based on 16S rDNA sequences. The sequence similarity was in the range of 95 to 99% to the described strains in database. All the thermophilic isolates resemble the genus, *Bacillus* including *B. thermoruber*, *B. methanolicus*, and *B. subtilis*. Phylogenetic analysis and physiological characterization of those strains were conducted.

Hyperthermophiles were isolated from deep-sea sediments of Bismarck Sea (03°18.971S, 152°34.843E; 1,650m) through Onnuri cruise in 2002. Hyperthermophilic archaeal culture NA001 and NA002 which can grow at 90°C are tentatively classified into the genus of *Thermococcus* and *Pyrococcus* respectively, based on 16S rDNA sequence data.

References

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Table 1. Defining conditions of deep-sea habitat and the implication for biotechnology^a

Habitat	Defining condition	Bioprospecting opportunities
Ocean trenches	High pressure	Novel and improved biocatalysts and chemistry
deep-seas, polar seas, cold seeps	Low temperature	Cold-active biocatalysts; bioremediation; surfactants; bioantifreeze
Seawater	Low nutrient concentration	High-affinity catalysts and ligands
Hydrothermal vents	High temperature, metals	Thermostable and solvent-stable biocatalysts; biohydrometallurgy
Sediments, epibioses, and symbioses	High nutrient concentration; defense mechanisms	Novel bioactive chemicals; sensing, signaling, and defense chemicals; consortia for enhanced turnover rates
Saturated brines	High salinity	Halotolerant biocatalysts; novel metabolites
Hydrocarbon	Hydrocarbons	Bioremediation, biotransformations
Deep sub-sea floor sediments	Anaerobic	Anaerobic biotransformations

^a From Bull et al (2000)

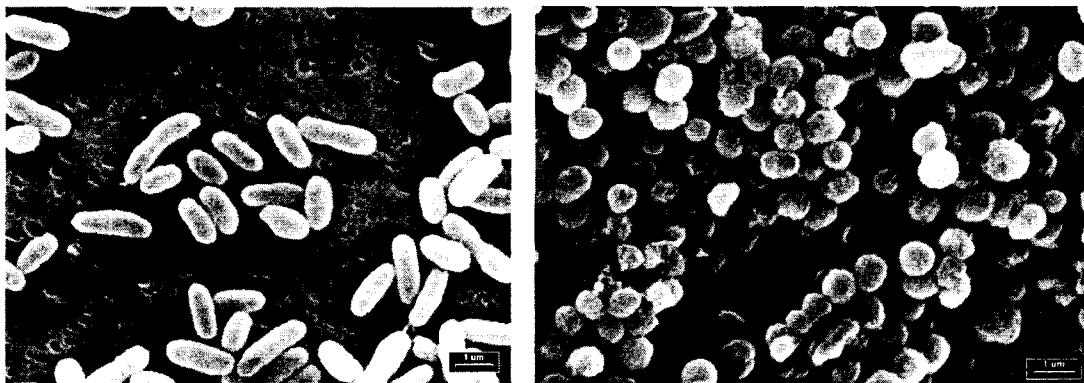


Fig. 1. Electron photomicrographs of a psychrophilic strain LT17 (A) and a hyperthermophilic strain NA1 (B) from marine environments.