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Ancient Sediment Harbors Remarkable Number of "Living" Microbes - NoanoSIMS Sheds Light on Life Deep under the Seafloor -

Overview

Scientists of Kochi Institute for Core Sample Research, JAMSTEC, and Atmosphere and Ocean Research Institute, the University of Tokyo found that a remarkable number of microbial cells are metabolically "alive" in deep and ancient subseafloor sediments off the Shimokita peninsula of Japan in the northern Pacific. The scientists examined the microbial incorporation of stable isotope-labeled substrates at single cell-level using nanometer-scale secondary-ion mass spectroscopy (NanoSIMS) and found that over 10 million cells per cubic centimeter of microbes from deep subseafloor sediment (*) were capable to assimilate carbon- and nitrogen-substrate at rates of one hundred thousand times slower than Escherichia.coli cells in the laboratory incubations. The results are the first time of quantitative evidence to show the metabolic potential of microorganisms in subseafloor biosphere, the most understudied ecosystem on Earth, This study is expected to lead to unveiling the role of subseafloor microorganisms on the global element cycle, evolution and adaptation in their environment.

The study was supported in part by the Japan Society for the Promotion of Science Funding Program for Next Generation World-Leading Researchers (NEXT Program). The work was published online on October 11 in the Proceedings of the National Academy of Science of the United States of America (PNAS).

*The sediment sample was obtained by the shakedown expedition of deep sea drilling vessel Chikyu in 2006, at a site approximately 80 km off the coast of the Shimokita peninsula (water depth: 1,180 m) from a depth of 219 meters below the seafloor (mbsf).

Title: Carbon and nitrogen assimilation in deep subseafloor microbial cells

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Background

Microorganisms living under the ocean floor are calculated to account for 10 percent of living biomass on Earth. Using the cores retrieved from the seafloor 80 km off the Shimokita peninsula, scientists tried to examine the metabolic activity of subseafloor microorganisms through their carbon and nitrogen metabolism -- a process indispensable to life.

Deep sea sediments have been attracting growing attention in recent years as host of next generation clean energy resources such as methane hydrate. Though the subseafloor microbes are believed to play an important role in producing such resources, it has been not clear even whether the cells are alive and metabolically active, or are dormant or perhaps dead, since their metabolic activities are extremely slow and difficult to be cultivated.

In this study, scientists examined carbon and nitrogen assimilation activities of the microorganisms in sediment slurries by supplementing 13C- and/or 15N-labled substrates (glucose, acetate, pyruvate, bicarbonate, amino acids, methane, and ammonia) as the traceable substrates. The assimilation amount and rate for each substrate were analyzed by NanoSIMS analysis (Fig. 1). The nanoSIMS measurement was carried out at the University of Tokyo and Institute Curie in France.

Results
Analysis using NanoSIMS showed that nitrogen was assimilated under all incubation conditions and carbon was assimilated in all except for those incubated with methane (Fig. 2). Approximately up to 76% of the microbial population incorporated the added substrate (Fig.3). This suggests that a remarkable number of microbial cells are remained "alive" in deep (219 mbsf) and old (460,000 years) sediments.

With regard to cell population, the image-based fluorescent cell enumeration showed that microbial cells grew on substrates when incubated with energy-rich organic compounds, which are glucose, pyruvate and amino acids. In contrast, incubation with acetate and bicarbonate, which are less energy efficient for cell growth, resulted in assimilation without supporting cell growth (Fig.3).

The rate of nutrient assimilation was estimated to be 10^{-16} (one ten quadrillionth) grams/cell per day, which is extremely slow compared with those of well-known, cultivated microorganisms (e.g. less than one hundred thousandth of E. coli).

In addition, the atomic ratios between nitrogen incorporated from ammonium and the total cellular nitrogen consistently exceeded the ratios of carbon, suggesting that subseafloor microbes preferentially require nitrogen assimilation for the recovery in laboratory (Fig.4). This indicates that microbial cells might control nitrogen uptake, conserve intracellular energy pools, and keep them alive for long periods of time in the nutrient scarce subseafloor sediment. These findings could open the possibly to clarify the evolution and adaptation of microbes in subseafloor biosphere.

Future studies
The method used in this study was a cutting-adge approach integrated approache of earth and biological sciences and expected to contribute to the advancement of interdisciplinary research into the biological, metabolic,

physiological and biogeochemical nature of subseafloor microbes. And this approach would help resolve material cycles in subseafloor environment (e.g. generation of natural resources), or examination and application of the capacity of subseafloor microbes for biogeo-engineering.

Figure 1. High Spatial Resolution Nanometer Secondary Ion Mass Spectrometry(NanoSIMS)
NanoSIMS is capable of visualizing isotope-labeled nutrient uptake by microbes at a spatial resolution as small as 50 nanometers. NanoSIMS instrument will be installed in JAMSTEC's Kochi Institute for Core Sample Research in December, 2011. One nanometer is one millionth millimeter.

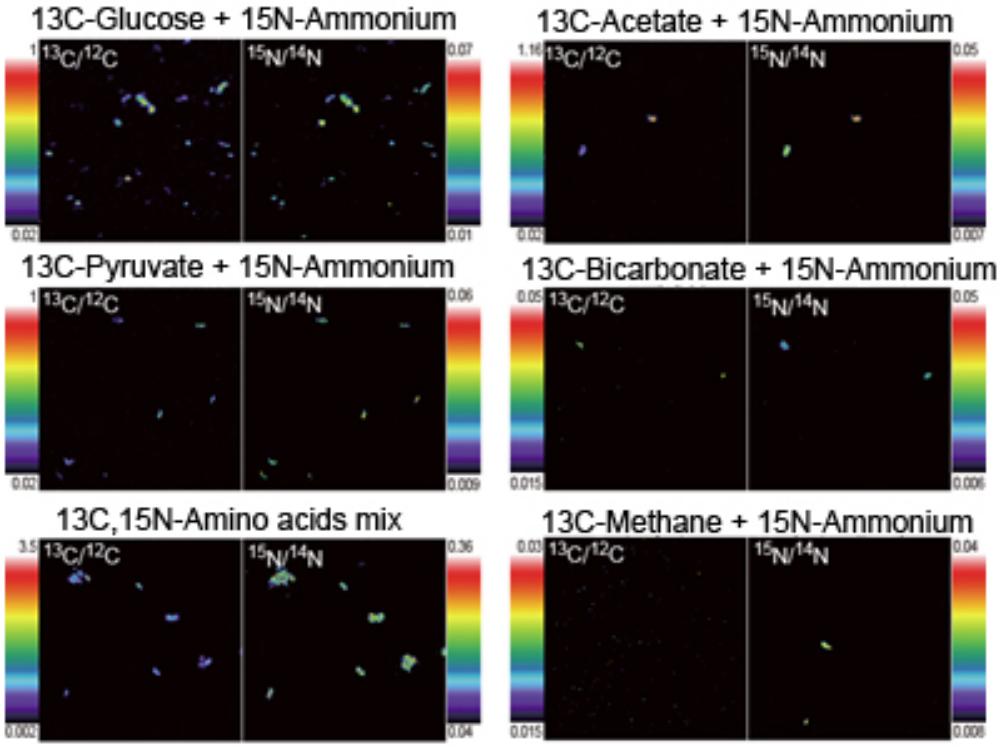

Figure 2. NanoSIMS images of subseafloor cells that incorporated stable isotope (13C and/or 15N) -labeled substrates. Color gradient indicates 13C or 15N abundance.
The size of microbial cells is 0.5 to 1 micrometers.

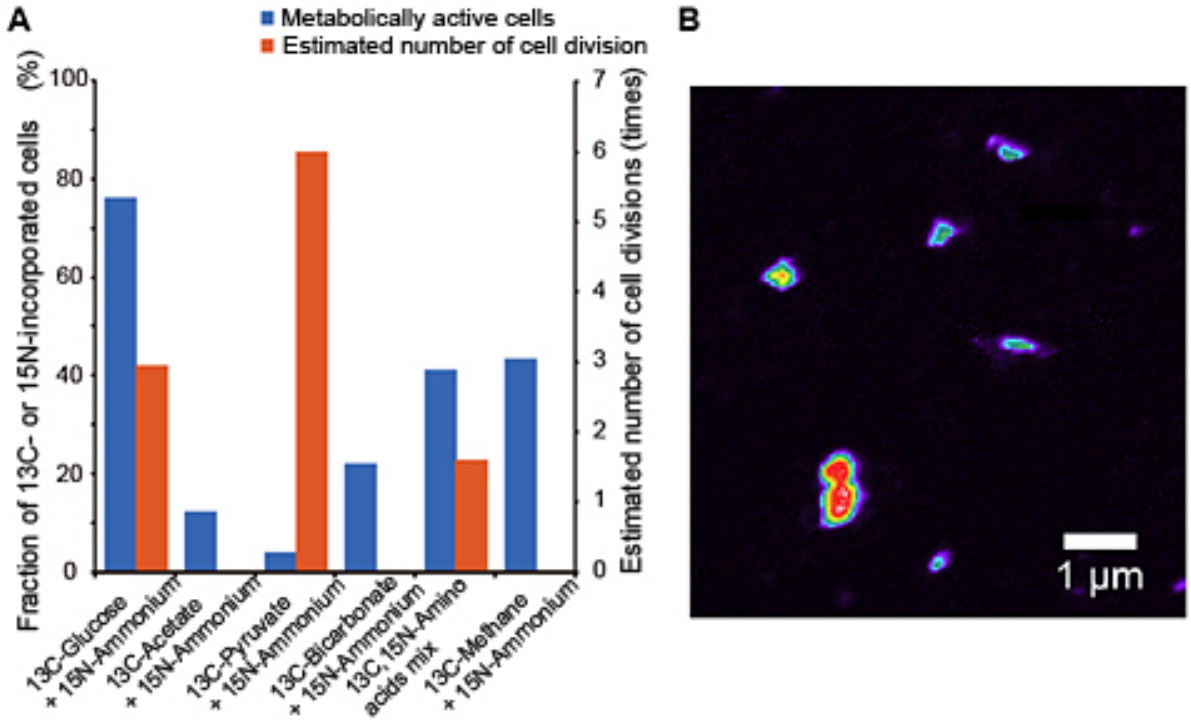

Figure 3. Carbon and nitrogen incorporation by subseafloor microbial cells from stable isotope-labeled substrates. (A) Fraction of nutrient incorporating cells (blue bars) and the estimated number of cell division (red bars). Increase in the number of cells was observed with glucose, pyruvate, or amino acids but not with acetate, bicarbonate or methane.
(B) Example of NanoSIMS image of microbial cells that incorporated 13C labeled glucose.

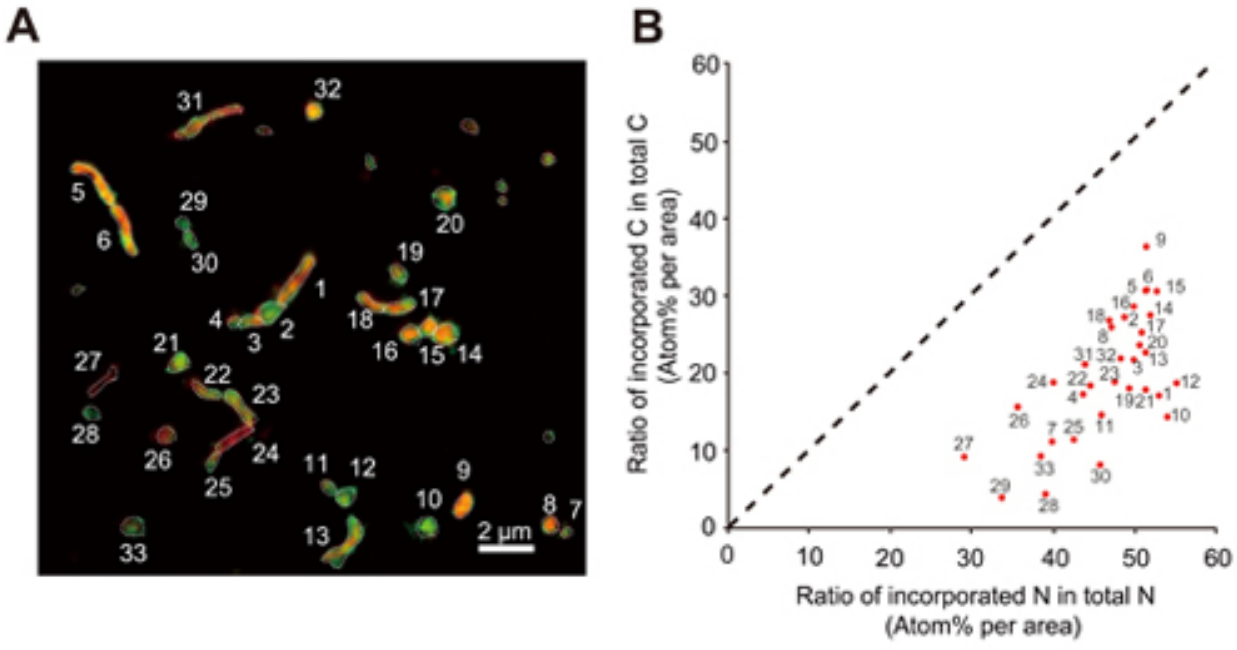

Figure 4. Localization of intracellular 13C and 15N incorporation in a deep subseafloor microbial community. (A) Example of overlaid ratio images of 13Cglucose (red) and 15N ammonium (green) visualized using NanoSIMS. (B) Scatter plot of atomic percentages of carbon and nitrogen incorporation in each of the numbered areas shown in A. The nitrogen incorporation ratios were higher than carbon incorporation ratios. Equal incorporation of carbon and nitrogen is represented by the dotted line.

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