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Transmission Electron Microscopic Observation of Drilling Microbiolgical Core Samples from a Deep Seafloor at Hydrothermal Vent Field

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Abstract - Autochthonous bacteria were found in clayey rocks below a deep sea floor in an active hydrothermal vent filed in the eastern Manus Basin, Papua New Guinea. Microbiological core samples were collected from Site 1188 of Leg 193 of Ocean Drilling Program (ODP). The amounts of carbon were quite low in all samples, nevertheless, the bacteria were directly observed on board without cultivation. Transmission electron microscopy revealed bacterial cooperation with clays minerals. Illite, and talc were present near the bacteria, indicating that clay minerals play an important role in the bacterial habitat, e.g., as a buffer against heat from hydrothermal fluid, and the change of water chemistry by the inflow, and as a sustainable food supply, such as H_2O and K^+. The findings of bacterial clayey community here add to the knowledge of subsurface biology in nm-order environmental system of life-chain.

I. Introduction

Since bacteria were found in igneous rock, the areas for research have largely expanded [e.g., 1-3]. These bacteria enter rocks from clacks that formed after magma cool dawn. If the location has no chance of the flow of nutrients, incidentally, the requirements for survival of the bacteria will be an autotrophic ability, or an enduring ability without nutrients for long period. Probably, bacteria in rocks with low nutrients must have habitat for bring above abilities into full play.

The PACMANUS hydrothermal system in the Manus back-arc basin is located at a water depth of 1700 m on the crest of a dacite volcanic ridge [4-5]. Leg 193 of the Ocean Drilling Program focused on active discharge sites reaching a maximum depth of 380 m below the sea floor. Fluid-dacite interaction generated secondary mineral assemblages, including argillaceous alteration and acid-sulfate alteration [7]. Pervasive and fracture-controlled fluid flow and overprinting alteration stages induced substantial textural modifications [8]. In this paper, one of the active discharge sites, Site 1188, was bio-clay mineralogicaly examined (Fig. 1). The forms and distributions of subsurface bacteria provided valuable information without cultivation for subsurface microbial ecology in hydrothermal systems of deep sea floors.

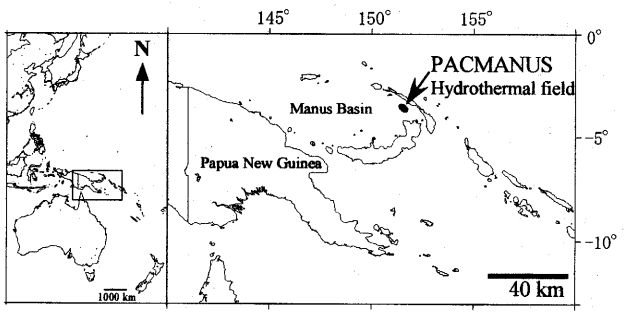

Fig. 1. Sampling locality map of the PACMANUS hydrothermal field of ODP Leg 193 in the eastern Manus Basin, Papua New Guinea.

II. Samples and Methods

Samples

Microbiological core samples were immediately selected from whole round drilling cores obtained on deck. The cores were handled with latex gloves that washed with 70 % ethanol to prevent contamination. After the outside of the cores was sterilized with a flame, the sample was transferred to an anaerobic chamber and equilibrated with an atmosphere containing a mixture of N_2 (90%), CO_2 (5%), and H_2 (5%), within 30 minutes of the on-deck arrival of the core. The entire outer surface of the cores was split off using a hydraulic rock trimmer to prevent drilling-induced contamination from the outer sample surface to the interior of the sample. The split interior parts were used for microbiological study. Additionally, two quantitative contamination tests, perfluorocarbon tracers (PFTs) as a tracer of drilling fluids and fluorescent microspheres with a similar approximate size to microorganisms (0.5 –1.0 μ m) as particles tracers, were conducted during the drilling operation [9-11]. The each test confirmed that the inside of the core was free of contamination [7], [12].

Methods

Optical microscopy

The samples crush and grind into pieces less than 100 μ m in size. The fine pieces (~1.0 mg) were placed on a glass slide and stained with 4', 6-diamidino-2-phenylindole (DAPI, 50 μ g/ml). During microscopic observation, the pieces were so thick that it was necessary to adjust the focus to observe all of the features. DAPI staining DNA in bacteria fluoresces blue under ultraviolet rays (wavelength: ~365 nm). On the other hand, some clay minerals with DAPI staining fluoresce yellow. Therefore, clay minerals were used to make it easier to observe the relationship between the bacteria and minerals.

Analyses of chemical compositions

The chemical compositions of microbiological samples were analyzed in order to better understand the relationships among the elements with depth. Microbiological samples were run on a NCS elemental analyzer (Amuko, NA2500NCS) for total nitrogen, carbon and sulfur. After air-drying, the sample powders (2 mg) were combusted at 1000 ℃ in an oxygen atmosphere. Sulfanilammide (C6H8N2O2S; C 41.84%, H 4.68 %, N 16.27 %, O 18.58 %, S 18.62 %) was used as the standard. The chemical compositions of microbiological samples with the exception of one sample (1188A 9.6-mbsf) were analyzed by an energy dispersive X-ray fluorescence spectroscopy (ED-XRF; JEOL JSX-3201). After air-drying, the sample powders were pressurized to change into pellets under vacuum condition. The pellets were measured using Rh-K α generated at 30 kV. Conversions of intensities into concentrations were accomplished using a standard less FP-Bulk program set in a computer. Major element oxides are calculated as a base of fluorescent X-ray generated from air-dried samples on the assumption that all elements are oxidized.

Mineralogical properties

Mineralogical properties were also analyzed by an X-ray powder diffractometer (XRD; Rigaku RINT2200) with Cu K α generated at 40 kV and 30 mA for investigating minerals attached to bacteria. After air-drying the sample, the powders were pressed to fit the diffractometer holding the sample. On the other hand, the XRD profiles of clay minerals were obtained from < 2 μ m clay fractions deposited on glass slides in order. Furthermore, a fine mist of ethylene glycol (EG) (15 % in water) was sprayed on dry clay fractions for supplemental information of clay mineral identification.

Transmission electron microscopy

For transmission electron microscopic observation (TEM; JEOL JEM-2000-EX), fine particles of the microbiological core samples (1188A 88.0- and 106.8-mbsf) were suspended in distilled water filtered using filter paper (0.2 μ m ϕ). A drop of the suspension was mounted on a micro grid. After air-drying, the uncoarted sample was observed at an accelerating voltage of 160 kV.

III. Results and Discussion

Optical and epifluorescence microscopic techniques revealed the existence of bacteria, such as cocci, bacilli, and actinomycetes (filamentous or dendritic bacteria) on board without cultivation (Figs. 2 and 3). Cocci and bacilli about 1.0 μ m in size was observed in the core of Hole 1188A 87.9-mbsf. Filamentous bacteria from 2.0 to 5.0 μ m in length were observed in the core of Hole 1188A 106.8-mbsf (Fig. 3A, B and C). The biofilms that developed consisted of a dense lawn of clay aggregates, each one of which contained one or more bacteria, phyllosilicates and grains of iron oxide material, all held together by bacterial extracellular polysaccharides [13].

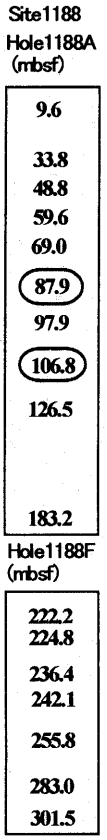

Fig. 2. Results of optical and epifluorescence microscopic observation of 17 microbiological core samples collected from Holes 1188A, and 1188F of Leg 193. Bacteria were found in 2 core samples (circles: presence of bacteria in cores).

The content of carbon in each sample ranges from 0.015 to 0.127 weight percent (wt %), with a mean value of 0.032 wt %. Nitrogen was not detected in any sample (detection limit: 0.01 wt%). The amount of carbon were quite low in all samples compared with the hydrothermally altered sediments found in the Middle Valley ([14]; Average: about 0.59 wt % total carbon and about 0.28 wt % organic carbon), and the deep-sea sediment samples from the Western Woodlark Basin ([15]; Average: about 0.58 wt % organic carbon). The depth profiles of carbon have no co-relationship with those of sulfur and iron (Fig. 4). It is possible that carbon and nitrogen limit the quantity of subsurface bacteria, even when the bacteria obtain metabolic energy from sulfur or iron. Other possibilities are that the permeability of hydrothemal fluid containing nutrients that

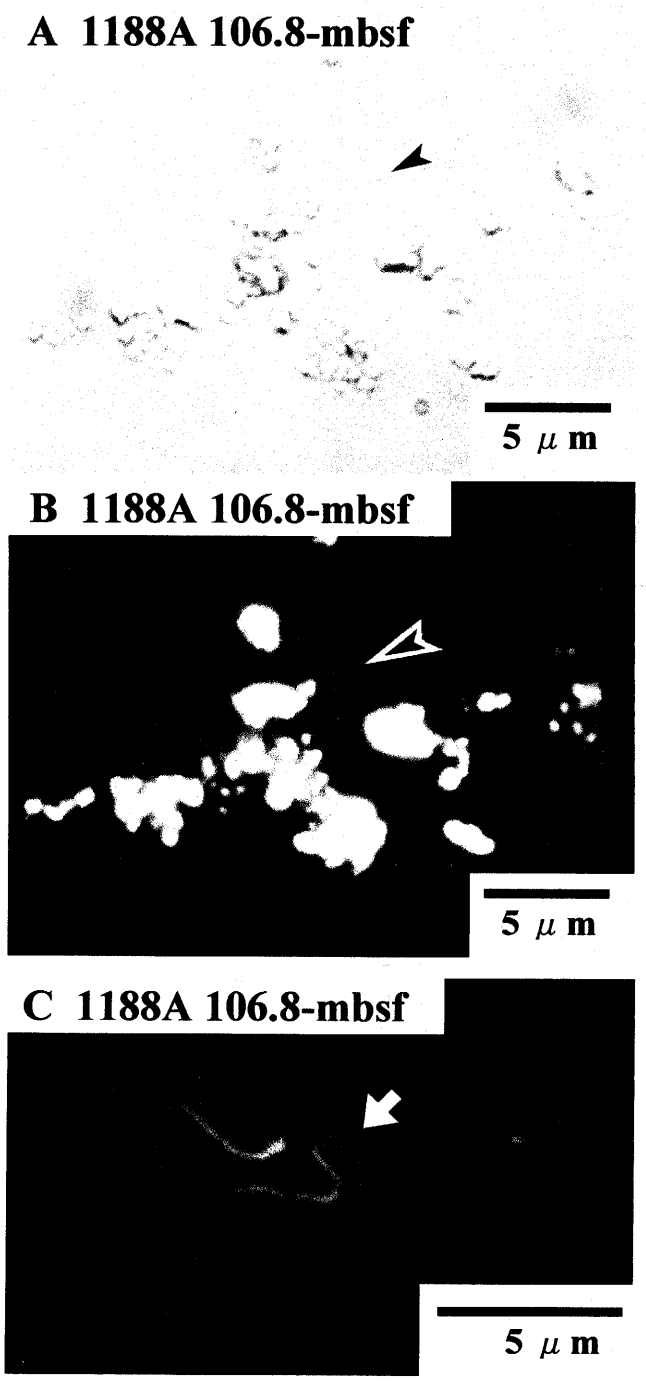

Fig. 3. Optical and epifluorescence micrographs of DAPI-stained samples collected from Hole 1188A 106.8-mbsf (A, B, and C). Arrows in micrographs indicate the presence of filamentous bacteria.

were derived from magma and/or seawater and the permeability may limit the quantity of subsurface bacteria in this area.

Holes 1188A and 1188F are only ~30 m apart, so together they provide a vertical section to a depth of 386.7 mbsf of the lithologic architecture. Volcanic rocks from the upper part are unaltered rhyodacite, and alteration varies with depth, and is complicated by overprinting relationships [7]. The core sample of 1188A 33.8-mbsf was rich in cristobalite and plagioclase, and did not contain clay minerals, and sulfide/ sulfate minerals (Table 1). Pyrite is the dominant sulfide in cores below 48.8-mbsf. In addition, microscopically, bacteria were not found in the euhedral anhydrite and pyrite. On the other hand, the core samples contain clay minerals, such as illite, chlorite, chlorite/semctite mixed layer, and talc. The results indicated that the minerals with fluorescent yellow found with bacteria under optical microscopy could be identified above clay minerals (Fig. 3). Clay mineral assemblage supplied a living space for bacteria, as it has been reported that clay minerals have important roles, e.g., as a buffer against heat from hydrothermal fluid, and the change of water chemistry by the inflow, and as a sustainable food supply, such as K^+ and H_2O (e.g., [13], [16-20]). Especially, illite intercalates K^+ of alkaline metal ion that works as a nutrient for microbes.

The microbiological core samples (Holes 1188A 87.9-mbsf and 1188A 106.8-mbsf) using transmission electron microscopy were observed. Two autochthonous bacteria in the range of 1.0 - 2.0 μ m in width were found with clay minerals in the core sample from Hole 1188A 87.9-mbsf (Fig. 5A). Cell walls of the bacteria were covered by flaky materials, and the internal cells are electrical high dense because of mineralization. The couple of bacteria were fragile by the convergence of the electron beam during observation. In addition, both bacteria were fragile by the convergence of the electron current during observation. The flaky material may be able to moderate excessive heat and precipitation from fluid flow. On the other hand, an autochthonous bacterium of 1.3 μ m in width were found with clay minerals in the core sample from Hole 1188A 106.8-mbsf (Fig. 5B). The bacterium has a thick cell wall with grid-like texture, and was covered by excretal exopolymers. The internal cell was also electrical high dense because of mineralization. Generally, the bacteria contained electron-transparent structures presumed to be carbon storage granule. But in Fig. 5 the bacterium contained mineralized material present in the cell.

At the hydrothermal site clay minerals are cradles of microorganisms to keep them. The clayey rocks may serve as carbon shelter. Such are the gifts of hydrothermal area, cradles of life in the deep seafloor. The findings of bacterial clayey community here add to the knowledge of subsurface biology in nm-order environmental system of life-chain. Further studies of subsurface communities below a deep seafloor in hydrothermal vent fields may not only provide many commercial products but also explain how life was before photosynthesis evolved. Such studies might also provide a new insight into whether microbes can live on the surface of Mars or some of the larger moons in the outer solar system.

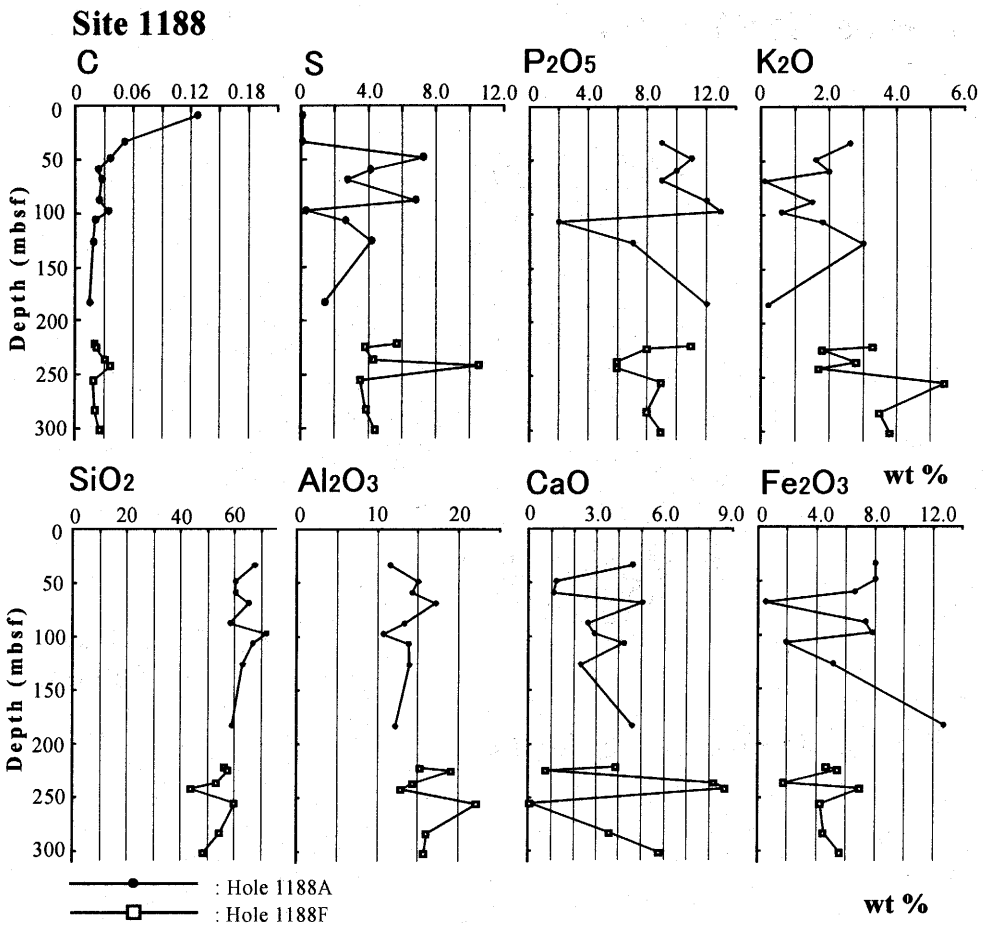

Fig. 4. Depth distribution of chemical components of total C, S, P_2O_5, K_2O, SiO_2, Al_2O_3, CaO, and Fe_2O_3 in microbiological core samples collected from Holes 1188A (●), 1188F (□). Carbon and sulfur were analyzed by an NCS elemental analyzer, whereas, P_2O_5, K_2O, SiO_2, Al_2O_3, CaO, and Fe_2O_3 were analyzed by ED-XRF spectroscopy. Note that the depth profiles of carbon have no co-relationship with those of sulfur and iron.

Table 1 XRD mineralogy of microbiological samples collected from Holes 1188A and1188F

Depth (mbsf)	Major minerals	Major clay minerals	Bacteria
1188A-			
9.6	n.d.	n.d.	
33.8	opaline silica, plagioclase, cristbalite	b.d.	
48.8	cristobalite, pyrite, quartz, gypsum	talc, illite	
59.6	cristobalite, pyrite, gypsum	chl/sm-mixed-layer, talc, illite, chlorite	
69.0	anhydrite, cristobalite, gypsum	talc	
*** 87.9**	**cristobalite, pyrite, gypsum, anhydrite, quartz**	**illite**	●
97.9	cristobalite, plagioclase, quartz, pyrite	chlorite, illite	
*** 106.8**	**quartz, anhydrite, gypsum, pyrite**	**talc, illite**	●
126.5	quartz, pyrite, anhydrite, plagioclase, gypsum	chl/sm-mixed-layer, illite, chlorite,	
183.2	quartz, plagioclase, cristobalite, pyrite	chlorite, illite	
1188F-			
222.2	quartz, anhydrite, pyrite	illite, talc	
224.8	quartz, pyrite	chl/sm-mixed-layer, chlorite, illite	
236.4	quartz, anhydrite, gypsum, pyrite	talc, illite	
242.1	quartz, anhydrite, gypsum, pyrite	talc, illite	
255.8	quartz, pyrite	illite, talc	
283.0	quartz, pyrite, gypsum,	illite, chl/sm-mixed-layer, chlorite, talc	
301.5	quartz, anhydrite, pyrite, gypsum	chlorite, illite	

n.d. : not determined, b.d. : below detection limit

* : Marked cores with an asterisk were observed by transmission electron microscopy.

● : Bacteria were found in marked cores by optical microscopy

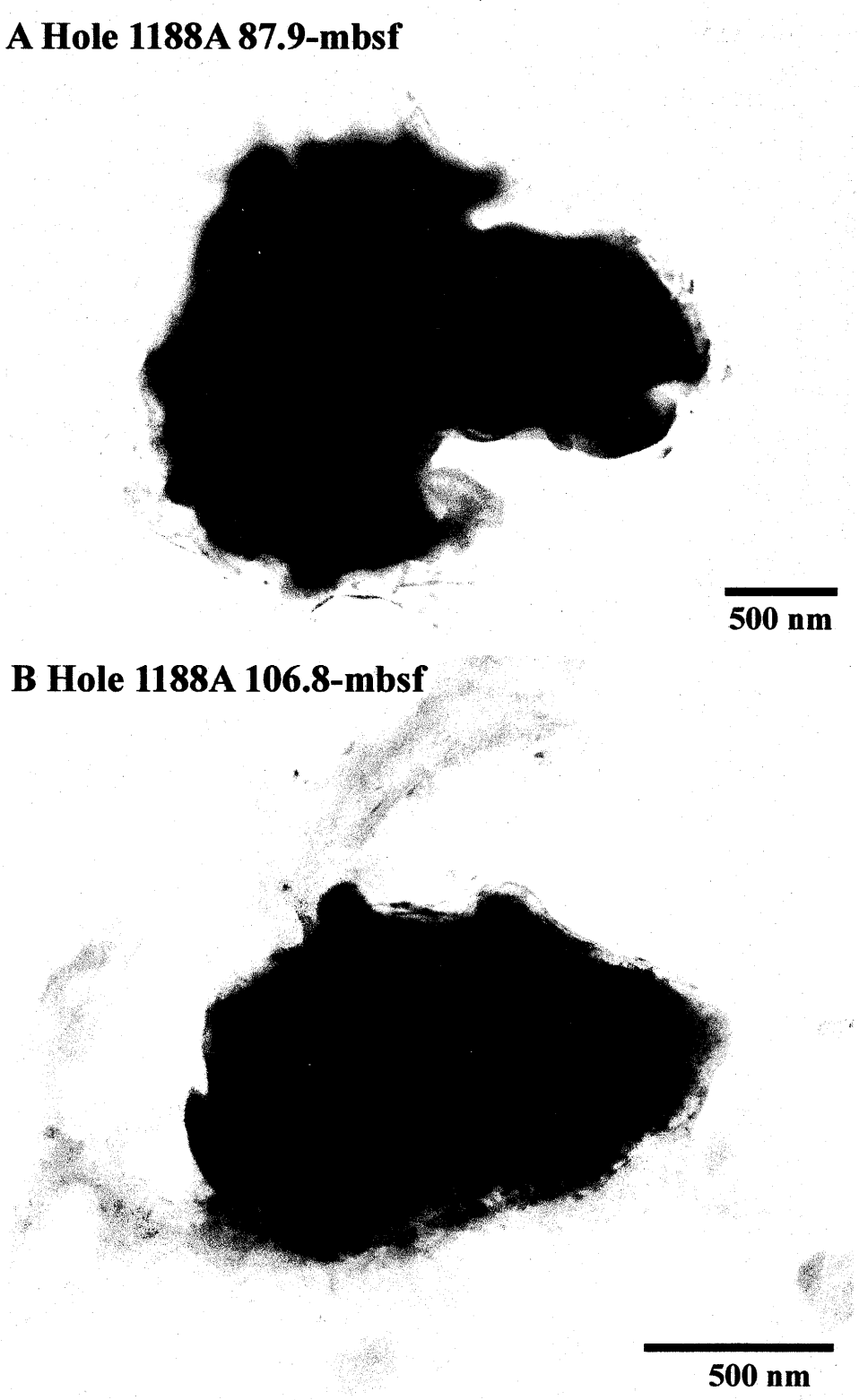

Fig. 5. Transmission electron micrographs of microbiological core samples collected from Hole 1188A 87.9-mbsf showing two bacterial cells in the range of 1.0 - 2.0 μ m in width (A) and from Hole 1188A 106.8-mbsf showing a bacterium of 1.3 μ m in width (B). These are autochthonous bacteria in the clayey rocks. (A): The cell walls were covered by flaky materials, and the internal cells are electrical high dense because of mineralization. The couple of bacteria were fragile by the convergence of the electron beam during observation. (B): The bacterium has a thick cell wall with grid-like texture, and covered by excretal exopolymers. The internal cell was also electrical high dense because of mineralization.

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