# Microbial biomineralization in weathered volcanic ash deposit and formation of biogenic minerals by experimental incubation

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# ABSTRACT

Microbial biomineralization in a weathered volcanic ash deposit from the 1914 to 1915 A.D. eruption of Sakurajima volcano was investigated by transmission electron microscopy (TEM) and energy dispersive X-ray analysis (EDX). The solution chemistry of pore water was also analyzed to elucidate saturation conditions. In addition, experimental incubations of bacteria collected from the volcanic ash were performed to confirm bacterial mineralization. TEM revealed that the weathered volcanic ash contains significant amounts of spherical to rod-shaped bacteria ranging from  $1.3 \times 10^8$ to  $2.6 \times 10^8$  cell/g, most of which have cell wall surfaces that are completely covered or decorated by either massive aggregates of allophane-like granular materials or irregular aggregates of smectitelike fibers and/or flakes. EDX confirmed that the granular minerals have chemical compositions similar to proto-imogolite allophane, whereas the smectite-like fibers and/or flakes show a wide range of chemical compositions corresponding to the compositional field between allophane and nontronite. The volcanic ash contains about 22 wt% of pore water, which is slightly acidic, relatively low redox potential, and enriched in Si, Na, Cl-, and SO<sub>4</sub><sup>2-</sup> ions. The saturation indices (SI) calculated by the PHREEQC geochemical code indicate that the pore water is almost saturated with respect to amorphous Al(OH)<sub>3</sub>, ferrihydrite, amorphous silica, and cristobalite, and significantly oversaturated with respect to silicate minerals in the order: halloysite < kaolinite < montmorillonite < allophane < nontronite. The allophane-like granular minerals seems to be preferentially precipitated by bacterial interaction with Al and Si ions in the pore water as a metastable phase. The poorly ordered smectite-like fibers and/or flakes may be transformed from the allophane-like materials as a intermediate phase between allophane and nontronite by the driving force originated from the greatest SI value of nontronite. The experimental incubation confirmed that amorphous silica containing a small amount of Fe is formed on the bacterial cell surfaces in liquid media with both Fe and Si ions. Likewise, beidellite-like smectite associated with the bacterial surfaces is produced in liquid media containing both Al and Si ions. However, no minerals are produced in the same media containing no metal ions or no bacteria. These results imply that bacteria play an important role in the accumulation of metal ions and in the formation of silicate minerals during weathering of volcanic ash.

# INTRODUCTION

Bacteria were the earliest cellular life of the Earth, appearing within the first billion years of the Earth's history (Cloud 1983; Ehrlich 1990; Chang 1994). Since then, bacteria have been widely distributed on the Earth's surface including soils, weathered rocks and sediments, lakes, rivers, and oceans. They are found from the atmosphere to both continental and oceanic deep crusts at temperature from freezing to boiling (Barns and

Nierzwicki-Bauer 1997). Geobiological studies have shown that bacteria play an important role in elemental circulation over the Earth's surface, especially through the interactions of cell wall surfaces with dissolved ions in the external environments producing various secondary minerals such as oxides, sulfides, carbonates, and silicates (e.g., Beveridge and Doyle 1989; Ehrlich 1990, 1999; Schultze-Lam et al. 1996; Konhauser 1998). This type of mineralization is called "biologically induced mineralization" (Lowenstam 1981), which proceeds extracellularly by the affinity of exposed functional groups of the cell surfaces such as carboxyl, hydroxyl, and phosphoryl groups for specific ions in the external fluid (Fein et al. 1997; Fowle

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and Fein 2000). The mass of minerals produced by such bacterial interaction over the Earth's history has been estimated to approach that of the total exposed land mass visible today (Beveridge and Fyfe 1985). The uppermost weathered layers of continental crust are known as major habitats of bacteria ranging from 10<sup>6</sup> to 10<sup>9</sup> cell/g, which is much higher than that found in freshwater and oceanic environments (Barns and Nierzwicki-Bauer 1997). Weathering environments are characterized by dissolution of rock-forming minerals and precipitation of secondary products. However, the effects of bacteria on the formation of silicate minerals in such weathering environments have not been well documented relative to other habitats such as acidic mine drainages, hot springs, and deep-sea hydrothermal vents.

This study investigates bacterial mineralization in a weathered volcanic ash deposit from the 1914 to 1915 A.D. eruption of Sakurajima volcano, Japan. We used mainly TEM and EDX to observe bacterial mineralization, and to analyze chemical compositions of the minerals associated with bacteria. Solution chemistry of pore water in the volcanic ash was also analyzed to verify the saturation conditions with respect to the minerals. In addition, experimental incubations of bacteria collected from the volcanic ash were performed in liquid media containing metal ions to confirm ion accumulation and mineral formation on the bacterial cell surfaces.

# MATERIALS

The Sakurajima volcano is located a few kilometers east of Kagoshima City in southern Kyushu, Japan. According to the historic record, the oldest eruption of the volcano took place in 708 A.D. Since then, there have been three large eruptions, i.e., 1471-1476 (Bunmei eruption), 1779-1781 (An-ei eruption), and 1914-1915 (Taisho eruption), producing huge amounts of lava flows and volcanic ash deposits (Aramaki et al. 1988). In 1955, volcanic activity recommenced with smoke and volcanic ash, and this has been maintained for 45 years. The Taisho volcanic ash deposit is widely distributed in north to east area of the volcano with a thickness of about 25 to 200 cm (Fig. 1). The Taisho volcanic ash for this study was collected from the sampling point situated about 2 km north of the volcano. In this area, ash thickness is about 2 m and overlies the An-ei volcanic ash. The upper 1 m of the deposit is composed mainly of fine particles of volcanic glass with lesser amounts of feldspar, pyroxene, and magnetite. The lower 1 m contains blocky pumice with a size of less than 5 cm, which tends to increase in size and abundance with depth. This deposit has no clear stratigraphic units, an is not clearly delineated. The ground surface of the Taisho volcanic ash deposit is covered by vegetation, but no development of soil profile was observed. The sample used here was collected from a trench with a depth of 15-20 cm, and was immediately sealed in a plastic bag to prevent biological contamination and evaporation of pore water.

# **EXPERIMENTAL METHODS**

XRD was conducted with a RIGAKU RU-200 diffractometer (CuK $\alpha$  radiation, 30 kV, 100 mA) equipped with a graphite monochrometer. XRD profiles were obtained from a powdered bulk sample loaded with a glass holder, and from <2  $\mu$ m clay

fractions deposited on a glass slide. The clay fractions were collected by sedimentation in distilled water and centrifugation. Quantitative estimation of constituents of the bulk sample was also carried out by internal standard methods using powdered sample mixed with a standard of NaCl.

SEM was performed with a HITACHI S-400 scanning electron microscope equipped with LINK ISIS EDX operated at an accelerating voltage of 15 kV using a carbon-coated sample. The chemical compositions of the bulk sample was determined with a EDX using a glass bead fused with  $Li_2B_4O_7$ . The chemical analyses of volcanic glass, feldspar, and pyroxene were also performed using hand picked grains placed on a carbon holder.

The volcanic ash contains significant amounts of pore water. The weight loss of about 10 g of volcanic ash sample after drying at 25 °C for 5 days was assigned to the pore water content. Pore water was extracted using a high-pressure press. About 300 g of the volcanic ash sample was placed into a stainless pressure vessel, and pressed slowly by oil pump at pressure up to 500 kg/cm<sup>2</sup>. About 15 mL of pore water was collected, and was used for chemical analysis after centrifugation to remove fine size contaminants. The solution chemistry of the pore water was analyzed by atomic adsorption spectroscopy for Al, total Fe, Na, K, Mg, Ca, and Mn, by calorimetric analysis for total dissolved Si, and by ion chromatography for F, Cl, NO<sub>3</sub>, and SO<sub>4</sub>. The content of Fe<sup>2+</sup> was also determined by the phenanthroline calorimetric methods. The measurments of pH and Eh values were made with a glass electrode of a digital pH



FIGURE 1. Map of Kagoshima Prefecture, southern Kyushu, Japan, showing the location of the Sakurajima volcano and the sampling point of volcanic ash used in this study. The sampling point is situated in about 2 km north of Sakurajima volcano. The darked area shows distribution of the Taisho volcanic ash deposit (1914 to 1915 A.D.) with a thekness of >25 cm.

meter. The temperature of the pore water was measured in the field. The saturation indices (SI) of the pore water were calculated by the PHREEQC geochemical code (Parkhurst and Appelo 1999) using the MINTEQ.DAT solubility database supplied with the PHREEQC package, supplemented with solubility data for allophane (Percival 1995).

TEM was carried out with a JEOL 2000 FX transmission electron microscope with LINK ISIS EDX operated at an accelerating voltage of 200 kV. Sample preparation for TEM involved mixing 1.3028 g of volcanic ash sample (= 1.0 g of air dried sample) with 100 ml of sterilized-distilled water. This mixture was ultrasonically homogenized for about 30 seconds (60 Hz, 40 W) and shaken for a few minutes, and then 0.005 mL of suspension, which corresponds to  $5 \times 10^{-5}$  g of air dried sample, was dropped on a Cu grid covered with a collodion film using a micro pipette. The Cu grid is 3.0 mm in diameter with 150 holes having a size of 0.117 mm, and the area ratio of hole/grid is approximately  $1.52 \times 10^{-3}$ . The assembly was carbon coated. The number of bacteria observed in the grid holes was directly counted under TEM. The total bacterial count in 1.0 g of sample was calculated from:

$$N = n/(5 \times 1.52 \times 10^{-8}) \tag{1}$$

where, N stands for total number of bacteria in 1.0 g of sample and n signifies the number of bacteria observed in each grid hole under TEM. Selected area electron diffraction was also conducted on secondary minerals using camera lengths of 1347 mm.

Bacteria were incubated on agar media. Five agar media containing 0.1 mM of NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub>, 1.0 mM of glucose, and 1.0 mg/L of yeast extract, with or without 1.0 mM of Fe, Al, and Si were prepared (Table 1). The medium lacking metal ions was used for the estimation of number of culturable bacteria in the volcanic ash. Media containing Al, Fe, and Si were used to test the effect of metal ions on growth of bacteria. To prevent precipitation of Al and Fe, EDTA (Ethylenediaminetetraacetic acid) was added as a chelating agent with the metal ions. The pH of each ager medium was adjusted to 6.0 using 10 mM NaOH or H<sub>2</sub>SO<sub>4</sub> solutions. The suspension of volcanic ash sample prepared for TEM was diluted with sterilized-distilled water for 10 times, and 0.1 mL of this diluted solution, which corresponds to 10<sup>-4</sup> g of air dried sample, was applied on the surface of agar media and spread homogeneously. Incubation was conducted at a constant temperature of 30 °C for a period of 30 days. During incubation, the number of bacterial colonies that appeared on the media surface were counted daily with the naked eye.

Formation of minerals on the bacterial surfaces was examined by incubation of bacteria in liquid media having the same compositions and pH as those of the agar media. The bacterial colony appearing on each agar medium was transferred and suspended in 25 ml of counterpart liquid medium. Each liquid medium was incubated in a rotary shaker at 120 r.p.m. under a constant temperature of 30 °C. After the 30-day incubation period, bacteria were separated by centrifugation and then thoroughly washed 3 times with sterilized-distilled water. This suspension was used for TEM after drying on a Cu grid covered with a collodion film, and for SEM-EDX after deposition on a carbon holder. Control experiments were conducted using the same liquid media without bacteria.

#### RESULTS

#### Mineralogy of volcanic ash

The volcanic ash is composed of 60-70 wt% volcanic glass, 15-20 wt% feldspar, 5-10 wt% of cristobalite, <5 wt% pyroxene, and trace amounts of magnetite and noncrystalline secondary products. The chemical compositions of the bulk sample (Table 2) is andesitic but enriched in Fe. The volcanic glass has a rhyolitic composition with minor variations in major elements. The feldspar (Table 2) is bytownite (Ab<sub>14</sub>An<sub>86</sub>). Two kinds of pyroxene having chemical composition of hypersthene (En<sub>61</sub>Fs<sub>39</sub>) and augite (Di<sub>60</sub>He<sub>40</sub>) are also present. The XRD profile of the bulk sample (Fig. 2) is characterized by a halo of volcanic glass that appeared over  $2\theta$  of  $15-35^{\circ}$  and by the sharp reflections of both feldspar and cristobalite. The <2 µm claysized fractions have a profile similar to that of the bulk sample, and there are no distinct reflections of crystalline clay minerals such as halloysite, kaolinite, and smectite (Fig. 2). However, it appears that the intensity of the volcanic glass halo increased relative to that of the feldspar reflections. This suggests that the relative percentage of volcanic glass increases with decreasing particle size.

#### Chemistry of pore water

The volcanic ash contains 23.24 wt% of pore water. The pore water is slightly acidic (Table 3), has relatively low redox potential, and is enriched in Si, Na, Cl<sup>-</sup>, and SO<sub>4</sub><sup>2-</sup> ions compared with those in average world river abundance (Berner and Berner 1987). However, the concentrations of Fe and Mn are low relative to the chemical composition of the volcanic ash, which may be due to processes such as oxidation, hydrolysis, and precipitation. Aluminum is the second major element of the volcanic ash, but its concentration in the pore water is very low. These major cations are possibly supplied from volcanic

TABLE 1. Chemical compositions of agar media used for culturing bacteria

Medium	NH <sub>4</sub> H <sub>2</sub> PO <sub>4</sub> (mM)	Glucose (mM)	Yeast extract (mg/L)	Fe (mM)	Al (mM)	Si (mM)	EDTA (mM)	pН	Incubation (day)	
No metal	0.1	1.0	1.0	-	-	-	-	6.0	30	
Fe	0.1	1.0	1.0	1.0	-	-	1.0	6.0	30	
AI	0.1	1.0	1.0	-	1.0	-	1.0	6.0	30	
Fe-Si	0.1	1.0	1.0	1.0	-	1.0	1.0	6.0	30	
Al-Si	0.1	1.0	1.0	-	1.0	1.0	1.0	6.0	30	

The chemical agents used as a source of AI, Fe, and Si are of  $AI_2(SO_4)_3$ ,  $Fe_2(SO_4)_3$ , and sodium silicate solution, respectively. EDTA was added as a chelating agent to prevent precipitation of metal-hydroxide. The pH was adjusted to 6.0 by using 10 mM of NaOH or  $H_2SO_4$  solutions.

 TABLE 2.
 Chemical compositions of bulk samples of the volcanic ash and its major constituents

				-		
	Bulk	Gls	Fld	Орх	Срх	
SiO <sub>2</sub>	62.81	72.91	52.19	52.66	53.37	
TiO <sub>2</sub>	1.05	0.94	0.05	0.25	0.65	
AI2O <sub>3</sub>	13.54	10.54	27.65	0.00	0.60	
FeO	10.22	5.36	1.30	23.76	13.92	
MnO	0.24	0.10	0.00	0.96	0.30	
MgO	0.15	0.00	0.00	20.76	11.99	
CaO	7.21	3.31	15.68	1.56	18.69	
Na₂O	2.35	3.82	2.71	0.00	0.00	
K₂O	2.44	3.02	0.42	0.05	0.48	
Total (%)	100.00	100.00	100.00	100.00	100.00	

Notes: Total Fe as FeO. The weight % of oxide compounds were normalized to 100% total. Bulk = bulk sample of weathered volcanic ash, Gls = volcanic glass, Fld = feldspar, Opx = hypersthene, Cpx = augite.



**FIGURE 2.** XRD profile of (**a**) bulk sample of volcanic ash and that of (**b**) clay sized fractions of <2  $\mu$ m in size.

ash by interaction with the pore water, but the Cl<sup>-</sup> and SO<sub>4</sub><sup>2-</sup> may be from new ash fall regime and/or from volcanic gases emitted by Sakurajima volcano because the amounts of SO<sub>2</sub>, N<sub>2</sub>, HCl, and HF discharged from the summit crater are estimated to be 1–2000 ton/day (Hirabayashi et al. 1982). The saturation indices (SI) calculated by the PHREEQC geochemical code (Table 4) for amorphous Al(OH)<sub>3</sub>, ferrihydrite, amorphous silica, and cristobalite are close to SI = 0, indicating that the pore water is almost saturated with respect to these minerals. In contrast, allophane, halloysite, kaolinite, montmorillonite, and nontronite show more greater SI values, suggesting that the pore water is significantly oversaturated with respect to these silicate minerals.

TABLE 3. Solution chemistry of the pore water

			) et ille pere	mator		
Si	0.8619	Mg	0.0933	F-	0.0121	
AI	0.1027	Ca	0.4074	CI-	0.6291	
Fe³⁺	0.0049	Na	0.8143	NO <sub>3</sub>	0.0126	
Fe <sup>2+</sup>	-	K	0.1117	SO <sub>4</sub> <sup>2-</sup>	0.8625	
Mn	0.0039					
	-	ture /°C		E 10 Eh	()) 0 11E U	

*Notes*: Field temperature (°C) is 24.2, pH = 5.19, Eh (V) = 0.115. Units are mmol/L.

TABLE 4. Saturation indices (SI) of the pore water

	( - )		
Minerals	SI	Minerals	SI
Amorphous AI(OH) <sub>3</sub>	0.27	Halloysite	6.20
Ferrihydrite	0.62	Kaolinite	9.47
Amorphous silica	-0.04	Montmorillonite	8.17
Cristobalite	0.53	Nontronite	18.50
Allophane	12.51		

Abiotic weathering of volcanic ash

Abiotic weathering of the volcanic ash proceeds mainly by interaction with water. This interaction causes dissolution of primary minerals, and then precipitates secondary minerals when the concentrations reach saturation. SEM observations revealed various surface micromorphology produced by dissolution. Most grains of feldspar and volcanic glass exhibit an angular blocky shape with flat surfaces, on which well-developed etch pits elongated in irregular directions are present. Similarly, pyroxene grains show blocky to rod-like morphology with lens-shaped etch pits aligned along [001]. The surfaces of feldspar and pyroxene grains, have some secondary minerals produced by precipitation. The most abundant secondary product is cristobalite as observed in the XRD profile (Fig. 2). The cristobalite exhibits roughly spherical-shaped morphology with a size of less than about 2.0 µm (Fig. 3a). EDX confirmed that the cristobalite grains contain mainly Si with small amounts of Na, Al, Ca, and Fe. The secondary abundant product is ferrihydrite which occurs as massive aggregates of fine particles on both feldspar and volcanic glass surfaces (Fig. 3b). EDX indicated that the ferrihydrite contains significant amounts of Si (Si/Fe = 0.17) and Al (Al/Si = 0.08) as frequently reported in natural and synthetic environments (Schwertmann 1988).

TEM observations revealed that the clay-sized fractions are composed mainly of volcanic glass and feldspar fragments, and very minor amounts of poorly ordered materials present as aggregates of randomly elongated fine fibers with a length of less than 0.5  $\mu$ m (Fig. 3c). Poorly ordered Al-silicates such as allophane and imogolite are absent in this sample. The electron diffraction patterns of these fibers display a diffuse halos, suggesting a poorly ordered structure. EDX indicated that the materials contain major amounts of Al, Si, and Fe with trace amounts of Ti and/or Mn. These poorly ordered Al-Si-Fe materials are known as a metastable phase appearing at the early weathering stages of volcanic glass, and may be transformed into more stable phases such as allophane and halloysite (Kawano et al. 1997; Kawano and Tomita 1999).

#### **Biomineralization in volcanic ash**

TEM observations show many spherical to rod-shaped bacteria which are mostly about 0.5 to 2.0  $\mu$ m in size. Most bacteria in the volcanic ash are surrounded by poorly ordered silicate minerals enriched in Al and Si, and variable amounts of Fe



**FIGURE 3.** SEM micrographs of (a) cristobalite and (b) ferrihydrite, and TEM micrograph with electronic diffraction pattern of (c) poorly ordered Al-Si-Fe minerals formed by weathering processes of the volcanic ash. The cristobalite exhibits a normally spherical-shaped habit with a size of  $<2.0 \,\mu$ m, whereas the ferrihydrite occurs in massive aggregated forms and contains small amounts of Si and Al. depending on their morphology. Figure 4a shows a bacterium exhibiting ellipsoidal forms of about 1.0 µm in size, which is surrounded by massive aggregates of very fine granular precipitates forming thin surface layer with a thickness of less than 0.1 µm. The electron diffractions of the precipitates display diffuse halos, suggesting a poorly ordered structure. From EDX, the massive aggregates contain mainly Al and Si, and small amounts of Fe, Ca, P, S, and Cl (Fig. 4b). Similar massive aggregates of granular precipitates on the bacterial surfaces are frequently observed, which are also characteristically enriched in Al and Si, and depleted in Fe. Other types of precipitates exhibiting irregular aggregates of fibrous and/or flaky minerals are more abundant. A typical precipitate of this type on the cell surface of a spherical bacterium is shown in Figure 4c. This bacterium is completely covered or decorated with aggregates of very thin fibers elongated in irregular direction and with no clear outline. The electron diffraction patterns of the fibrous minerals exhibit diffuse halos with no lattice fringes, suggesting a poorly ordered structure. From EDX, the minerals contain mainly Al, Si, and Fe with minor amounts of P, Ca, and Ti (Fig. 4d). Figure 5 shows the same type of precipitate exhibiting well-developed fibrous and/or flaky forms that produced around the cell surface of a spherical bacterium having rod-shaped protuberances. The electron diffractions of the fibrous and/or flaky minerals confirmed also a poorly ordered structure, and revealed that the minerals contain mainly Al, Si and Fe.

The chemical compositions of the poorly ordered minerals associated with bacteria have a wide range of chemical compositions (Fig. 6), ranging from 0.65 to 3.43 for Al/Si, and from 0.02 to 1.11 for Fe/Si. The massive precipitates tend to have relatively larger Al/Si and smaller Fe/Si ratios than those of the fibrous and/or flaky precipitates. These chemical compositions differ from those of crystalline Fe-clay minerals such as chamosite, nontronite, glauconite, and celadonite, and are occur in compositional field between proto-imogolite allophane and nontronite (Fig. 6).

## Number of bacteria in volcanic ash

TEM showed that each grid hole has about 10 to 20 cells of bacteria. The total number of bacteria was estimated as approximately  $1.3 \times 10^8$  to  $2.6 \times 10^8$  cell/g from Equation 1. The number of bacterial colonies that appeared on the medium without metal ions (Table 1) increased rapidly after 3 days, and reached more than 300 within 10 days, and plateaued at about 330 (Fig. 7). From this count, the volcanic ash contains at least  $3.3 \times 10^6$  cell/g of culturable bacteria, which is one to two orders of magnitude smaller than the TEM count. Such discrepancies have been frequently reported, and it is well known that the number of culturable bacteria is less than 10% of the total bacterial population in soils (Tate 1995).

The number of bacterial colonies that appeared on the agar media with 1.0 mM Fe and Al reached about 240 and 140, respectively, after 30 days. These numbers are less than those from medium without metal ions, suggesting that bacterial growth is inhibited by the presence of Fe and Al ions. When adding 1.0 mM Si ions, the number of colonies decreased significantly relative to no Si counterparts, indicating further inhibition on bacterial growth by Si ions.



(Cu) 2 8 10 0 2 8 10 ENERGY / KeV ENERGY / KeV

FIGURE 4. TEM micrographs and EDX spectra of bacteria surrounded by (a, b) massive aggregates of granular minerals and (c, d) irregular aggregates of fibers and/or flakes.

# Experimental formation of bacterially mediated minerals

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To demonstrate the formation of minerals by bacterial mediation, experimental incubations in liquid media with or without metal ions were performed using bacteria collected from colonies that appeared on the agar media. All experimental runs in liquid media with 1.0 mM Fe produced noncrystalline spherical materials with a diameter of less than 0.1 µm around the bacterial cell surfaces as observed by TEM (Fig. 8a). EDX indicated that the spherical materials contain mainly P and Fe, and significant amounts of K and S (Fig. 8b). This Fe-phosphate material has a Fe/P ratio of approximately 1.0 and includes a minor amount of K, which is very close to leucophosphite (KFe<sub>2</sub>(PO<sub>4</sub>)<sub>2</sub>OH·2H<sub>2</sub>O; Lindsay et al. 1989). The

bacterial incubation in liquid media with 1.0 mM Al formed noncrystalline needle to rod-shaped materials with a length of less than 1.0 µm, most of which occurred on the bacterial surfaces or as aggregates associated with bacterial cells (Fig. 8c). This material is enriched with S, Na, and Al, and also contains variable amounts of Cl and K as detected by EDX (Fig. 8d).

For liquid media with both Fe and Si, TEM reveals a bacterially mediated precipitation of amorphous silica exhibiting aggregates of fine particles which covered the surface of rodshaped bacteria (Fig. 9a). The precipitates are highly enriched in Si and depleted in Fe relative to the solution composition (Fig. 9b). This indicates that the bacterial cell surfaces have a strong affinity for Si, thus promoting chemical binding and



**FIGURE 5.** TEM micrograph with electron diffraction pattern and EDX spectrum of a spherical bacterium with well-developed noncrystalline Al-Si-Fe minerals on the cell surface.

precipitation of Si rather than Fe. This observation conforms to the favorable binding of Si with the bacterial cell walls relative to other metal ions (Urrutia and Beveridge 1993, 1994). For the liquid media with both Al and Si, well-developed aggregates of thin fibers and fine particles were produced around the bacterial surfaces during 30 days incubation period (Fig. 9c). The electron diffractions of the fibrous materials display hexagonal spot patterns coinciding with those of smectite. EDX indicates that the minerals consist mainly of Si and Al, with small amounts of Na and S (Fig. 9d). This is also similar to chemical composition of smectite containing S impurity.

These bacterial mineralizations are observed in all liquid media with metal ions, but no minerals are produced throughout the incubation period in the liquid media without metal ions or bacteria (Table 5).



**FIGURE 6.** Ternary diagram showing distribution of Fe, Si, and Al in the poorly ordered minerals associated with bacteria, with plots of generalized compositions of some related clay minerals. Open circles = compositions of the massive aggregates of granular minerals. Filled circles = compositions of the irregular aggregates of fibers and/or flakes. Stars = generalized compositions of some related clay minerals, recommended from Nomenclature Committee as follows: Be = beidellite [Na<sub>0.33</sub>Al<sub>2</sub>(Si<sub>3.67</sub>Al<sub>0.33</sub>)O<sub>10</sub>(OH)<sub>2</sub>], Ce = celadonite [KMgFe<sup>3+</sup>Si<sub>4</sub>O<sub>10</sub>(OH)<sub>2</sub>], Ch = chamosite [(Fe<sup>3+</sup><sub>3</sub>Al)(Si<sub>3</sub>Al)O<sub>10</sub>(OH)<sub>8</sub>], Gl = glauconite [K<sub>0.85</sub>(Fe<sup>3+</sup>, Al)<sub>1.34</sub>(Mg,Fe<sup>2+</sup>)<sub>0.66</sub>(Si<sub>3.76</sub>Al<sub>0.24</sub>)O<sub>10</sub>(OH)<sub>2</sub>], No = nontronite [Na<sub>0.33</sub>Fe<sup>3+</sup><sub>2</sub>'(Si<sub>3.67</sub>Al<sub>0.33</sub>)O<sub>10</sub>(OH)<sub>2</sub>], Pha = proto-halloysite allophane (Al/Si = 1.0), and Pia = proto-imogolite allophane (Al/Si = 2.0).



**FIGURE 7.** Numbers of bacterial colonies appearing on the agar media with and without metal ions during incubation for 30 days.



**FIGURE 8.** TEM micrographs with electronic diffraction patterns and EDX spectra of (**a**, **b**) bacteria surrounded by Fe-phosphate produced during experimental incubation in the liquid medium containing Fe for 30 days, and (**c**, **d**) bacteria associated with Na-sulfide containing small amounts of Al formed in the liquid medium containing Al during experimental incubation.

FIGURE 9. TEM micrographs with electronic diffraction patterns and EDX spectra of  $(\mathbf{a}, \mathbf{b})$  bacteria surrounded by amorphous silica with small amounts of Fe produced during experimental incubation in the liquid medium containing Fe and Si for 30 days, and  $(\mathbf{c}, \mathbf{d})$  bacteria with smectite formed in the liquid medium containing Al and Si during experimental incubation.

#### DISCUSSION

#### Weathering conditions of volcanic ash

Weathering of volcanic ash proceeds firstly by dissolution of primary materials such as volcanic glass, feldspar, and pyroxene, and followed by precipitation of secondary products from solutions containing dissolved ions. Therefore, the solution chemistry is the most important factor in formation of secondary products of such weathering environments. The SI values calculated by the PHREEQC indicate that the pore water of the volcanic ash is almost saturated with respect to amorphous Al(OH)3, ferrihydrite, amorphous silica, and cristobalite, and significantly oversaturated with respect to allophane, halloysite, kaolinite, montmorillonite, and nontronite (Table 4). However, the volcanic ash contains ferrihydrite, cristobalite, and poorly ordered Al-Si-Fe minerals as secondary products with no crystalline phase. This observation is consistent with the short time of weathering period (84 to 85 years). As is well known, amorphous Al(OH)3, ferrihydrite, amorphous silica, and cristobalite tend to precipitate as a metastable phase in the early stages of weathering, and are transformed eventually into a stable crystalline phase by subsequent reactions (Williams et al. 1985; Williams and Crerar 1985; Schwertmann 1988; Kawano et al. 1997). Thus, ferrihydrite and cristobalite observed in the volcanic ash must be directly precipitated from the pore water as a metastable phase. The ferrihydrite contains significant amounts of Si (Si/Fe = 0.17). This is attributed to a strong affinity of Si ions for hydroxy Fe ions which leads to the formation of Fe-silicate minerals such as Si containing ferrihydrite (Karim 1984; Vempati and Loeppert 1989). The pore water is also saturated with respect to amorphous Al(OH)3, but poorly ordered Al-Si-Fe minerals are present while amorphous Al(OH)<sub>3</sub> is not. Al ions are known to produce hydroxy Al-silicate ions in solutions containing both monomaric Al and Si, and to precipitate poorly ordered Al-silicate minerals when saturation of Al is achieved (Luciuk and Huang 1974; Wada and Wada 1980). Furthermore, Fe ions are readily incorporated into the structure of Al-silicate precipitates (Farmer et al. 1991a). Hence the poorly ordered Al-Si-Fe minerals may be formed instead of amorphous Al(OH)<sub>3</sub>.

#### Characterization of microbiologically mediated minerals

It is known that great amounts of bacteria exist in weathering environments of rocks and sediments, but their effects on ion accumulation and mineral formation have not been well documented. This study characterizes properties of bacterial mineralization in the weathering environment of volcanic ash. (1) The total number of bacteria in the volcanic ash of be  $1.3 \times 10^8$  to  $2.6 \times 10^8$  cell/g, is similar to bacterial count in weathered sediments such as soils (Barns and Nierzwicki-Bauer 1997). (2) Most of the bacteria in the volcanic ash are completely covered or decorated with aggregates of poorly ordered silicate minerals around the cell wall surfaces. These precipitates can be divided into two types morphologic types: one is massive aggregates of granular minerals, and the other is irregular aggregates of fibers and/or flakes. (3) The minerals associated with bacteria are composed mainly of Al, Si, and Fe with a wide range of Al/Si and Fe/Si ratios ranging between compositions of proto-imogolite allophane and nontronite. However, the massive aggregates can be characterized by relatively larger Al/Si and smaller Fe/Si ratios, whereas, the fibrous and/or flaky aggregates tend to have smaller Al/Si and larger Fe/Si ratios.

Characteristics of other environments are as follows. For example, bacteria produce ferrihydrite-like poorly ordered Feoxides with occasional presence of goethite and jarosite in acid mine drainages (e.g., Ferris et al. 1989; Bigham et al. 1990; Mann et al. 1992; Fortin and Beveridge 1997). These minerals are known to be produced by biochemical oxidation of Fe(II) (Colmer and Hinkle 1947). On the contrary, amorphous silica and/or Fe-oxides containing variable amount of Si are the most abundant products in hot springs (Ferris et al. 1986; Schultze-Lam et al. 1995; Hinman and Lidstrom 1996; Jones and Renaut 1996), and also near deep-sea hydrothermal vents (Fortin et al. 1997, 1998). Calcite is another predominate mineral in hot spring environments, which is often formed on the cell surfaces of cyanobacteria (Tazaki et al. 1995; Jones and Renaut 1996; Fouke et al. 2000). In contrast, poorly ordered Al-Si-Fe minerals with a chamositic composition are predominately formed as authigenic minerals on the bacterial surfaces in freshwater environments such as rivers and lakes (Ferris et al. 1987; Konhauser et al. 1993, 1994, 1998). The bacterially mediated minerals in the volcanic ash studied here closely resemble the microbiogenic minerals found in freshwater environments rather than those in acidic mine drainages and hot springs. Similar poorly ordered Al-Si-Fe minerals associated with bacteria are known to be widely produced in weathering environments of other pyroclastic deposits containing slightly acidic pore waters (Kawano and Tomita 2001). Such observations possibly suggest that this type of bacterial mineralization may be common and widespread in weathering environments of rocks and sediments.

# Formation of Al-Si-Fe minerals on the bacterial cell surfaces

Association of poorly ordered Al-Si-Fe minerals predominantly with the bacterial surfaces indicates that these minerals form induced by bacterial interaction with dissolved cations such as Al, Si, and Fe as reported in other natural environments. The bacterial cell walls and surrounding extracellular slimes are constructed of negatively charged organic polymers containing carboxyl, hydroxyl, and phosphoryl groups, which are highly interactive with solutes, especially with metal ions in aquatic environments (Beveridge 1981, 1989; Fein et al. 1997). It seems likely, therefore, that the organic polymers act as a chemical adsorber and also as a template for precipitation and growth of the poorly ordered Al-Si-Fe minerals. Similar strong ability of ion accumulation has been confirmed by cell wall fragments of dead bacteria (Beveridge and Koval 1981; Beveridge and Fyfe 1985). Thus, the bacterial surfaces have the ability to precipitate minerals, but the process may depend on the solution chemistry of surrounding environments. Conversely, bacteria promote highly efficient mineral precipitation leading to formation of a poorly ordered metastable phase, but formation of a final stable phase is controlled by the equilibrium principles as well as abiotic mineralization.

Two types of poorly ordered Al-Si-Fe minerals exist in the volcanic ash. Chemical compositions of the allophane-like granular precipitates are very close to proto-imogolite allophane (Al/Si = 2.0), whereas those of smectite-like precipitates have a wide range of compositions between proto-imogolite allophane and nontronite. Possibly, these two types represent different stages of mineral formation from a poorly ordered metastable phase to a crystalline stable phase. Allophane-like materials tend to precipitate rapidly in solutions containing Si, Al, and Fe ions by incubation at room temperature with and without bacteria, but more longer time is required for formation of layer silicate minerals (Farmer et al. 1991a, 1991b; Urrutia and Beveridge 1994, 1995). Thus, the allophane-like granular precipitates seems likely to be an early stage product of bacterial interaction, whereas the smectite-like precipitates having a wide range of chemical compositions may be a intermediate phase between the allophane-like materials and nontronite. The SI of nontronite shows greater value than that of allophane, indicating a stronger driving force on nontronite formation. However, the faster formation rate of allophane relative to nontronite causes first precipitation of the allophanelike materials, and they will eventually transform into nontronite through a poorly ordered intermediate phase. In this formation process, the precipitation of allophane-like materials may also be promoted by bacterial interaction with dissolved ions in the pore water.

# Experimental formation of bacterially mediated minerals

Incubations of the bacteria in liquid media indicate that the bacteria accumulate metal ions and produce some minerals on their cell surfaces depending on the metal ions present. From Table 5, Fe-phosphate and Na-sulfide containing Al are produced by incubation with Fe and Al, respectively, at pH 6.0 condition (Fig. 8). At this pH, Fe and Al are present as monovalent anions of Fe-EDTA and Al-EDTA chelate compounds, and P and S ions exist as mainly monovalent  $H_2PO_{\overline{4}}$  anions with small amounts of HPO<sub>4</sub><sup>2-</sup> and divalent SO<sub>4</sub><sup>2-</sup> anions, respectively (Lindsay 1979). It has been assumed that these anions interact with positively charged sites such as amine groups situated throughout the cell wall matrix (Urrutia and Beveridge 1994). In the case of liquid media containing Fe, P would be possibly bound to the positively charged sites and then reacts with Fe-EDTA to produce Fe-phosphate. Likewise, the binding of S to the positively charged sites promotes the precipitation of Na and Al, and the subsequent growth of Na-sulfide containing Al in the liquid media with Al.

For the liquid media with both Fe and Si, amorphous silica containing a small amount of Fe is produced on the bacterial surfaces (Fig. 9a, b). In this pH condition, Si ions are mainly present as neutral  $H_4SiO_4$  ions with trace amounts of monovalent  $H_3SiO_4^-$  anions (Iler 1979), whereas Fe ions occur as monovalent anions of Fe-EDTA chelate compounds (Lindsay 1979). The Si-rich and Fe-poor product indicates that the bacteria preferentially accumulate Si relative to Fe on their cell surfaces. Similar behaviors of preferential precipitation of Si over Fe have been observed on the cell surfaces of *Thiobacillus*, and two possible hypotheses explaining the preferential pre-

TABLE 5. Biogenic minerals produced by bacterial interaction with various metal ions during experimental incubation in liquid media for 30 days.

	-			
Medium	Metal ions in media	Minerals produced by bacteria		
No metal	No metal ions	No minerals		
Fe	1.0 mM Fe	Fe-phosphate (leucophosphite?)		
AI	1.0 mM AI	Na-sulfide containing Al		
Fe-Si	1.0 mM Fe + 1.0 mM Si	Amorphous silica containing Fe		
Al-Si	1.0 mM AI + 1.0 mM Si	Smectite		
No bacteria	Metal ions + No bacteria	No minerals		
Notes: The chemical compositions and pH of the liquid media are the				
same as those of the agar media listed in Table 1. Incubation was con-				
ducted by using a rotary shaker at 30 °C for 30 days.				

cipitation of Si have been proposed (Fortin and Beveridge 1997). First, there is the binding of small amounts of Si anions to positively charged sites and subsequent nucleation. Second, there is the binding of metal cations to negatively charged carboxylate or phosphorylate and the resulting complexes serve as bridges to bind Si anions. In the present experiments, it is more likely that the amorphous silica would be precipitated by the reaction involving the first hypothesis rather than the second one, because Fe ions are present as monovalent anions of Fe-EDTA chelate compounds, which prevent binding of Fe to the negative sites.

Relatively large amounts of Al and Si are precipitated to form beidellite-like smectite on the bacterial surfaces in the liquid media containing both Al and Si. In this case, formation of smectite is possibly induced by binding of Si to the positive sites of bacterial surfaces in a similar manner as in Fe-Si system. The large amounts of Al are then incorporated with Si polymers during precipitation and subsequent crystallization. The equilibrium constant of Al-EDTA is about eight orders of magnitude lower than that of Fe-EDTA (Lindsay 1979), and ionic affinity between Si and Al ions to produce hydroxy complex is apparently stronger than that between Si and Fe (Iler 1979). These ionic properties may promote incorporation of Al ions into the structure of Si polymers.

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