Abstract
Large amounts of microfossil records discovered in the seafloor black smoker chimney are reported from the Okinawa Trough. They are well preserved and can be divided into four types of filamentous microfossils. It suggests that the fossils may be derived from sulfur or iron oxidation chemolithotrophic prokaryotes and fungi. Based on the comparison studies of the microbial mineralization processes, two steps of biomineralization were hypothesized: (1) biology controlled mineralization; and (2) biology induced mineralization. At the early stage of the mineralization, the biology controlling mineralization is dominating; at the later stage, the biology inducing mineralization is the main mechanism. The composition of the fluids and the species of the microbes will determine the types of the minerals formed.

Key words: microfossils, biomineralization, black smoker, Okinawa Trough

1 Introduction

The discovery of submarine hydrothermal systems and the associated biological communities is one of the most important scientific achievements in the studies of global oceanic geology and biology in recent years. The discovery of submarine hydrothermal systems has changed our previous understanding of marine geology to some extent. The extreme circumstance where the hydrothermal biological communities exist around black smoker is quite distinct from the continental and known shallow sea biological communities so that it is an ideal lab to explore the origin of life. Therefore, researches on black smoker have significant implications to both the submarine mineralization and the origin of life (Feng et al., 2004; Scott, 2002; Cook and Stakes, 1995; Fouquet et al., 1993, 1991; Haymon et al., 1989). The study of the fossilization of hydrothermal microorganism and the mechanism of mineralization will not only help to understand the submarine hydrothermal activities, but also open up the windows to access more details on identifying the lives in ancient petrology and minerals on other planets in the universe.

The Okinawa Trough, a young outspread basin, is an important part of the circum-pacific volcanic and earthquake belts, where it is involved in frequent tectonic activities and volcanism. Its special geographical location and geological phenomena have attracted researchers worldwide. Thermal activities have been discovered and hydrothermal biological communities have been observed mostly in the middle section of the Okinawa Trough since 1984 (Kimura, 1988). During several scientific explorations of the submarine of the Okinawa Trough from 1992, we have studied the submarine hydrothermal biological community (Jiang, 1998; Gao and He, 1996; Ma and Zai, 1996) besides submarine hydrothermal deposits and mineralization. The main studies have covered breed distribution, searching for foods, ecotypic habits and so on, however, the studies of thermopiles microorganism around hydrothermal spout and bacterial fossils are rare. Iheyabideg is located in the middle of the Okinawa Trough, which is NNW-SSE inclined rectangular valley with 6 km in length, 3 km in width and 1 250–1 610 m in depth (Zeng et al., 2001; Gao and He, 1996). The sulphide black smoker was obtained at a depth of 1 300 m below water surface in the Okinawa Through.

Series researches have been conducted on the submarine black smoker from the Okinawa Through (Feng et al., 2006). These researches focus on the characteristic shape, composition, texture and structure of the mineralization microorganism in black smoker and
also conduct a primary discussion on the mineralization process by microscope, SEM, Back-scattered, and X-ray diffraction.

2 Texture and structure of black smoker

Black smokers in the Okinawa Trough are in conical and/or cylindrical shapes (Figs 1a, b, c, d), which tend to be convergent upward or become spout structure on the top. The chimneys can be divided into two parts: ekteine and channel (kernel). Some of the chimneys preserve channel structures, which are aligned around the geometric axes. Mineral zones and texture zones are observed along the channels. Quite idiomorphism zinc blende, chalcopyrite, galenite, iron pyrites can be seen in the channel while the ekteine is composed of amorphous silica, blende, copper prites, iron oxide, and hydrate, among which are porous. Some chimney (Fig.1a) has been filled by idiomorphism zinc blende, chalcopyrite, iron pyrites, carbonate and thus perfect shape is conserved. All samples have clear zones and the porous structure change to branching porous structure from ekteine to the central channel.

Fig.1. Samples of black smoker in the Okinawa Trough.

As indicated by the results of the Rigaku DMAX 2400 X-ray diffraction from the National laboratory of Rare Earth Materials Chemistry and Application, the major mineral composition of the submarine black smokers in Okinawa Trough idiomorphism zinc blende, chalcopyrite, galenite, iron pyrites and rare galena.

3 Major microorganism fossils types and characteristics in black smoker

The black smoker samples from the hydrothermal activity areas in the Okinawa Trough, demonstrate typical channel structures. By microscope and high distinguish ability SEM, we discovered plenty of mineralized filaceous microorganism fossils, which can be separated into four types in the following according to the size and shape:

Type 1: Vertical rod, 4 μm in diameter, uniform width, 10-80 μm in length, the longest rod is over 300 μm in length. The filaceous fossils are in dense filament shapes, and tend to intergrow with other types of filaceous thus forming sheeted texture (Fig. 2). Sometimes it is single and is covered by many globoids with a diameter of 4-5 μm (Fig. 3). These rods are generally hollow and bifurcate. Outer tube surfaces are silicified at large. The surfaces of some stalks are smooth, while some are knurly. The cores of most stalks are hollow, the ends are sphericity, and the transects are
rotund.

Type 2: These are filaceous fossils, some of which are ramiform. They are uniform in a diameter of 5 μm and length of 20–80 μm. They are straight, curved or twisted. Different structures intersect and intertwist, which result in a sheeted texture. (Fig. 4d). The amount of this structure could be so large and numerous that forms a mat-like structure, which is the major structures observed in the samples. These filaments are hollow with the ektexine having turned to siliconised ektexine. The diameter of the core, most of which is sulfide, is about 250 nm. Some of the filaments are too small in diameter to be identified by XPS. We find most of them are idiomorphism galenite, iron pyrites and some amorphism as bearing sulfide (Figs 4a, b, c). Strumaeae are widespread on the surface of these filaments. The ends are spherical while the sections are irregularly rotund.
Fig. 4. Types of filaments 2. a. The thickness of filaments is well-distributed. They cross or twist in different direction to form mat-like structures (micrograph); b. filaments show tubular construction, the walls of which have been siliconised. The siliconised core consists of galenite, iron pyrites and amorphous sulfide containing arsenic (micrograph); c. SEM images. The surfaces of filaments (white arrows) are bumpy, the head of which is spherical and transverse sections are irregularly rounded; d. SEM images. Filaments (white arrows) form mat-like structures in high densities in inclusions.

Type 3: Helical filaments. This type is 4 μm in diameter, with hollow pipe and sphenicity end, silicon ektexine, smooth surface and no sulfide mineralization at the core. This kind of filament occurs less frequent than other kinds and tends to intergrow with straight stalks (Fig. 2).

Type 4: Fossils in high density and intertexture and intertwist in different directions, Fossils are 2 μm in diameter and more than 100 μm in length with rounded and smoother surface. No silicified ektexine (Fig. 5).

Fig. 5. Types of filaments 4 (micrograph).
It is very difficult to find organic bodies in filaments because the organic bodies are oxidized easily when the cells of microorganism are transformed into fossils (Hofmann and Farmer, 2000), thus the identification of microorganism fossils is much more difficult and that has induced many disputes. Once the bacteria, either from the nature or the laboratory, are mineralized, the fossils maintain similar texture, size, shape, cell complexity, tube of cells texture and the habit of the origin microorganism (Westal et al., 2001). The shapes of cells control the size and shape of microorganism fossils including sphericity, rod, filaments, leptospiro and stalked structure and so on (Southam and Donald, 1999). Therefore, these characteristics may provide marks for identifying the microorganism fossils (Westal et al., 2001). The identification mark of hydrothermal microorganism fossils (identified By optical or high-resolution microscope, such as Scanning electron microscope) mainly include size, special shape (including spherical, tubal, curly, helical and stalked structures), the complexity of cells cleavage, community gathering and biology films and indirect ones such as biology corrosion (Feng et al., 2005).

The filaments mentioned above are bended, divaricated, intercrossed and intertwined. Some have obvious disseipements while some others are spherical on the end with rounded or ellipse cross sections than irregular shapes due to the appearance of strumae on the surface (Fig. 6). Each type of microorganism appears in communities and presents mixed intergrowth. Abiogenical filaments do not demonstrate uniform diameter or tubular filaments (Banfield et al., 2000). These texture characteristics (such as size, shape and gathering) indicate that the fossils are biologic genesis and mineralized microorganism fossils. The microorganism can form different sheeted texture while growing. Both current and gravity can pose impacts upon the direction of filaments. The coupling of filaments in sheeted texture indicates that it is overlaid by gravity. The filaments are composed of non-rigid and soft minerals, such as organic polymers, and in the initial stages appear the characteristics covered by gravity. The typical characteristics of microorganism, that is tubular texture (with aperture of 1.5–1.1 μm) and sheeted texture, indicate that they are soft at the beginning of coupling. These filaments are similar to microorganism fossils found in hot spring of the deep sea in shape and texture. Therefore, our conclusion is that they are mineralized microorganism fossils.

![Fig.6. Silicified filaments in lead and vanadium.](Image)

Type 1 filaments tend to be in large diameters. They are not only long but also have divarications, which indicates that they might be filamentous epi-phyte; the shape of helix filaments in Type 3 is similar to iron oxide bacterium Gallionella; filaments in Types 2 and 4 have divarication, which implies that they are related to chemotrophic microbial life or archaea because eubacterial lineages don’t have divarication while growing. Filaments bacterium belongs to sulfide in configuration taxology, which is the primary producer in hydrothermal biogeoconose (Juniper and Fouquet, 1988). Filaments might be the characteristic phenomena in the sulfide fluid. The microorganism, which consists of the filaments at the boundary layer of onflow μ, has an important function in adjusting the local environment around (Taylor and Wirsen, 1997).

4 Microorganism mineralization process in black smoker

Microorganisms ingest hydronium from the en-
vironment. The ingested hydroium may deposit insid-
or outside of the cells or arise through chemical re-
tions. This process is defined as “Biomineralization”.
Formation of at least 250 minerals are related to biomi-
neralization (Kazue, 1995; Lowenstam, 1981).
There are two types of biomineralization: biologi-
ically-controlled mineralization (BCM) and biologi-
duced mineralization (BIM) (Lowenstam, 1981).

Biologically induced mineralization is not domi-
nated by microbes. Microbes only involve in chang-
ing the environment of mineralization, such as chemi-
cal elements of medium, pH value and the conditions of
redox. Minerals are generally crystallized outside of
cells with various granularities and shapes. Crystals
could contain impurity or form glomerocryst. Coral
reef is a typical example of this type of mineral. On
the other side, biologically-controlled mineralization
is controlled by microbes entirely. Minerals formed gen-
erally take on own attribution. Articles of minerals are
generally formed inside of cells with distinct shapes.

Marine hydrothermal activity can form Fe-sulfide
deposit, the formation of which is closely related with
the thermophiles activity. Hydrothermal minerals not
only deposit at spout of high temperature, but also
low temperature (Fortin et al., 1998). Numerous sam-
ple of iron oxide/silica material from sites on the East
Pacific Rise, Juan de Fuca/Explorer Ridges, and other
areas are porous and consist of branching filaments of
iron oxide and amorphous silica.

Take Philosopher Vent on Explorer Ridge in the
northeast Pacific as an example where hydrothermal
fluid was discharged at 27 °C from a chimney, 1.5 m
in height. The fluid was enriched with iron and sil-
ica over ambient seawater, but contained no H₂S. The
major constituents of the chimney were amorphous
silica (opala, 73%), and iron oxide (7.0%). Micro-
scopic examination revealed that the chimney consists
of hollow filaments, 1–2 μm in diameter. This evi-
dence combined with the presence of organic carbon
(1.3%), gives us an opportunity to investigate how the
microorganisms involve in the mineral precipitation.
We present here results of morphological and miner-
alogical investigations of samples from both oceanic
and terrestrial locations, and offer an assessment of
biological and non-biological mechanisms that could
have formed these deposits. Filamentous bacteria are
very abundant around hydrothermal vents, which can
urge the minerals to crystal and gather. The exis-
tence of organic matters and filamentous bacteria in-
dicates that the deposit of minerals is related with fila-
mentous microorganism (Juniper and Fouquet, 1988).
Samples collected in low-temperature waters near hy-
drothermal vents of the Southern Explorer Ridge, in
the northeast Pacific Ocean, contained fine Feand Mn-
oxide and Fe-silicate particles coating bacterial sur-
faces. Partially or totally mineralized bacteria, along
with bacterial exopolymers, were covered with a mix-
ture of poorly ordered Si-rich Fe-oxides (possibly fer-
rihydrite), Mn-oxides, and Fe-silicates (possibly non-
tronite). Minerals occur as very fine (2–20 nm) gran-
ular material, fine (20–100 nm) needles and sheets,
small (200–500 nm) nodules and filaments (i.e., min-
eralized exopolymers).

The pilot study indicates that the microbial min-
eralization in the modern black smoker from the Ok-
inawa Trough and the Mesoproterozoic black smoker
fossils discovered from Gaobanhe in the eastern Hebei
has mainly two types as follows.

(I) The metal ions can get into the cells dur-
ing frequent contacts between microbes and the hy-
drothermal fluids (Fortin et al., 1994). Metal reaction
is formed by-products or by the direct transformation
of metals by bacteria (Gorby and Lovley, 1992). The
axis of filamentous is firstly mineralized to amorphous
sulfide containing arsenic and iron. As soon as the
cells are dead or degraded, the metal ions associated
with the cell membranes can become the cores of min-
eral crystals, which promote the sulfide mineralization
within the filamentous structures.

Because hydrothermal fluids is not oversaturated
with amorphous silicon, during the mixture process of
pure hydrothermal fluids and sea water, amorphous
silicon can not be formed. The best way of forming
amorphous silicon deposit is to prevent the mixture of
pure hydrothermal fluids and sea water. Partial re-
friération can form numerous amorphous silicon de-
posits, which will increase the liquor saturation not sil-
icon. The increase of density and thickness of bacteria
and epiphyte may prevent the mixing of hydrothermal
fluids and sea water which consequently increase the
liquor saturation and may also mediate mineral pre-
cipitation (Pracejus and Halbach, 1996).

While organic groups are released, the siliceous
precipitate is likely to form immediately at the cell-
water interface, outlining and eventually encapsulat-
ing the organism. This embedding process could be
sufficiently slow for a continuous growth and multi-
plication of the microbial cells, otherwise life would
not be able to survive over geological time spans in
such a particular environment. At present, we are not
able to decide whether the organism will also incorporate silica into the cell walls to increase the rigidity of the membranes. The smooth appearance of the filaments, however, might indicate this possibility, because silica polymers in the cell walls could act as nucleation sites for further organogenesis silica precipitation; the inorganic spherical silica precipitation was also observed in our samples as a characteristic contrast with the mineralized filaments both in shape and size. Abiogenically amorphous silicon is formed at the last stage which covered the former minerals including mineralized protonema. Some of the filaments are too small in diameter to be identified by XPS. Surface of abiogenical deposited silicon is generally rough; on the contrary, the biologically genetic silicon has a smooth surface. These mineralized microbes and intercellular polymer will be covered with silica minerals which could possibly wrap the articles of abiogenical sulfide. Bacteria function as the solid reactant during geochemical reactions. They can also accelerate the mineral depositing (Konhauser et al., 2001; Konhauser, 1998). Later on, amorphous silicon replaced (Fig. 7b) by sulphide absorbs a lot of galenite articles on the surface (Fig. 7c).

Fig. 7. Types of mineralization I.

(II) Two mechanisms of absorption onto cellular surfaces of these microorganisms have been previously identified. It is because that bacteria wall assumes net-negative charge and contain amidogen and carboxyl which can absorb various hydronium and minerals to bacteria walls. Negative ion on the surface of cells has strong sorption to minerals. Binding metals and negative ion is possible during the first step of mineralization, but nucleation of minerals only happens when hydrous environment surrounding cells reach over saturation. The microbial cells can extract mineral crystals from the hydrothermal fluids. Metals’ binding and deposit on the surface of cells can increase the thickness of metals. Sulfide mineralization is firstly provided to the wall of cells. After the individual microbial cells have become entombed and begin to degrade, organic matter will be relics. Dead cells can bind more metals and negative ion than living cells (Urrutia et al., 1992). These poorly crystallized phases can reorder and become more crystalline with time. These mineral nuclei are stabilized by the wall and are less prone to dissolution because the wall reduces the interfacial tension between the mineral nucleus and the bulk water phase. They can gradually become crystal cores, and function as the solid reactant during geochemical reactions. They can also increase the mineral depositing speed. Mineral growth then is most active at the outer surface of the bacterium where space constraints by the envelope polymers do not inhibit metal precipitation (Fig. 8b).

Fig. 8. Types of mineralization II.
5 Conclusions

Plenty of microfossils were found in the seafloor black smoke samples from Okinawa Trough. They are preserved in good shapes, main of which are four kinds of filaments. The filaments show different shapes, such as, curl, divaricating, cross, twist. Some have apparent septum. Transverse sections are round or oval. Others are bumpy because they are coated with spherical and sub-spherical beads of amorphous silica. In rare examples there is an open central tube. Each of them presents itself in communities, which indicates that they exist in a symbiotic relationship. The structure characteristic of the filaments indicates that they are formed by living things and they are mineralized microfossils.

The study shows that two steps of biomineralization were hypothesized based on the comparison studies of the microbial mineralization processes: (1) Biology-controlled mineralization: The metal ions can get into the cells during the frequent contact between microbes and the hydrothermal fluids. Metal reaction is formed as by-products or by the direct transformation of metals by bacteria. The axis of the filament is firstly mineralized to amorphous sulfide containing arsenic and iron. At the same time, the increase of density and thickness of bacteria and epiphyle may prevent hydrothermal fluids and sea water mixing to increase the liquor saturation and may also mediate mineral precipitation. These mineralized microbes and intercellular polymer will be covered with silica minerals. Later on, amorphous silicon replaced by sulfide absorbs a lot of golenite articles on the surface. As soon as the cells are dead or degraded, the metal ions associated with the cell membranes can become the cores of mineral crystals, which promote the sulfide mineralization within the filamentous structures. (2) Biology-induced mineralization: Two mechanisms onto cellular surfaces of these microorganisms have been previously identified. It is because bacteria walls assume net-negative charge and contain amido-gen and carbxyl and absorb various hydronium and minerals to bacteria walls. Metal ion can stick or deposit on the surface of the cells, which would increase the metal concentration on the cell surface. Sulphide mineralization is firstly provided to the wall of cells. After the individual microbial cells have become entombed and begin to degrade, organic matter will be relics. However, these poorly crystallized phases can be reordered and become more crystalline with time. These mineral nuclei are stabilized by the wall and are less prone to dissolution. They can also become crystal cores gradually, and function as the solid reactant during geochemical reactions. They can also increase the mineral depositing speed. Mineral growth then is most active at the outer surface of the bacterium where space constraints by the envelope polymers do not inhibit metal precipitation.

The pilot study indicates that such microorganism in black smoker in the Okinawa Trough not only lives on marine hydrothermal activity, but also plays an important role in hydrothermal mineralization. Though studies by now haven’t completely made certain the detailed process of bacteria promoting hydrothermal mineralization (Fortin et al., 1998), the effect of bacteria on hydrothermal mineralization is not unimportant. Deep studies of the structure, minerals constitute, and mechanism of mineralization of microorganism fossils will impel further understanding of the relationship of microorganism activity and hydrothermal mineralization as well as the mechanism of mineralization.

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