

## Mineral phase of COCs and fibers in coral skeletons

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**Abstract.** The variation in the Sr/Ca ratio of coral skeletons is sensitive to changes in temperature and presents an archive for reconstructing the paleo-seawater environment. Information on the mineral phases corresponding to coral skeletal textures from micro- to nano-meter scales would be helpful toward resolving the mechanism causing the variation of Sr/Ca ratio. To confirm mineral phase differences in coral skeletons, we conducted the mineral phase identification in microstructures and nano-textures of the coral *Porites lobata* by X-ray diffraction analysis with synchrotron radiation (SR-XRD) and transmission electron microscope (TEM), respectively. Coral skeletal microstructures consist of centers of calcification (COC) and fibers. COCs are < 30  $\mu\text{m}$  in diameter in the samples. The X-ray beam size of SR-XRD collimated of 15  $\mu\text{m}$  and 40  $\mu\text{m}$  in diameter enabled us to obtain the information of mineral phase in COCs and fibers separately. All diffraction peaks of COCs and fibers can be explained as aragonite with the lower detection limit of ca. 0.5 wt% of strontianite by SR-XRD. All TEM observations also indicate the aragonite phase in coral skeletal nano-textures.

**Key words:** Mineral phase, SR-XRD, Coral, Skeleton, Microstructures

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### Introduction

The strontium/calcium (Sr/Ca) ratio in coral skeletons varies with surrounding temperature change and thus may provide an accurate paleo-thermometer of sea surface temperature (SST) (de Villiers et al., 1995). Coral skeletons are reportedly composed of only aragonite so that the Sr heterogeneity derives from the compositional rates of solid solution of aragonite.

Gregor et al. (1997) reported that as much as 40% of strontium (Sr) in coral aragonite existed as strontianite ( $\text{SrCO}_3$ ) by using X-ray absorption near-edge structure (XANES) and extended X-ray absorption fine structure (EXAFS). The presence of strontianite in coral skeletons could significantly influence Sr/Ca paleo-thermometry because the thermometry assumes the strontium in coral aragonite exists in solid solution. Gregor et al. (1997) implied that Sr did not substitute simply for Ca, and therefore coral Sr/Ca thermometry could not be applied for paleo-environmental analysis. Indeed, coral skeletons contain higher amount of Sr than expected by the solubility limits calculated from synthetic aragonite (Plummer and Busenberg, 1987). On the other hand, EXAFS analyses performed by Finch et al. (2003a, 2003b) and Allison et al. (2005) showed no evidence of the presence of strontianite or its intermediate state in coral aragonite. However, neither XANES nor EXAFS can identify the mineral phase directly.

Recent advances of micro analytical methods allow the analysis of trace elements within the coral skeletal

micrometer scale structures (microstructures) that correspond to daily growth resolution (Meibom et al. 2008). Cohen et al. (2001) focused on the substantial heterogeneity of Sr/Ca components in coral skeletal microstructures, which were not solely temperature related. Previous studies explained this heterogeneity as resulting from biological effects, kinetic effects, and different mineral phases. However, no model has yet been generated to explain the effects of biological, kinetics and mineral phases on the observed chemical and isotopic variations. Identification of the mineral of the coral skeletal microstructure and nano-meter scale structures (nano-textures) are thus crucial for explaining these effects.

Few studies on the mineral phase in coral skeletal microstructures have been performed (Vongsavat et al. 2006, Stolarski et al. 2007, Przenislo et al. 2008). Stolarski et al. (2007) were the first to report the mineral phase of coral skeletons, extracted as *en bloc* without pulverization, by high-resolution synchrotron radiation diffraction. However, their study did not analyze the mineral phase corresponding to coral skeletal microstructures.

Here we report the mineral phase identification in coral skeletal microstructures and nano-textures using X-ray diffraction analysis with synchrotron radiation (SR-XRD) and transmission electron microscope (TEM). Microstructures of coral skeletons consist of two morphology; COC (centers of calcification) and fibers (fasciculi) (Partz, 1882; Ogilvie, 1886). The SR-XRD analysis method enables one to identify the

mineral phase of COC and fibers by X-ray spots, 15  $\mu\text{m}$  and 40  $\mu\text{m}$  respectively. Our study provides the first analysis of the mineral phase in coral skeletal microstructures and nano-textures.

### Material and Methods

All data were obtained from the scleractinian coral *Porites lobata* collected from Ishigaki Island, Okinawa prefecture, Japan in 2005. The coral samples were prepared by first removing the tissue layers with water and allowing to dry at ambient temperature. Each sample was then cut, rinsed with distilled water and dried at room temperature. Samples were embedded in epoxy resin (Logitech Ltd.) and polished.

Microstructures in the coral skeleton were examined using scanning electron microscopy (SEM) in a section cut vertical to the axis of growth direction on a HITACHI S-3000H at Hokkaido University. Acceleration voltages of the electron beam were 10kV. All samples were polished and etched with 0.5M HCl (5-second), then sputter-coated with a thin layer of platinum.

We performed SR-XRD analysis to identify the mineral phase in coral skeletal microstructures. Before the SR-XRD analysis, we ensured the analysis area was free of micro-holes, then bonded the epoxy resined coral skeletons to the glass slide and polished them < 100  $\mu\text{m}$  thickness. In previous studies, the samples could not be separated from slide because of the thinness of the sample. All the samples have been prepared under the room temperature and were peeled from the glass slides. The experiments were conducted with an imaging plate (IP) on BL-18c at Photon factory, KEK (high energy accelerator research organization), Tsukuba, Japan. A monochromatic incident X-ray beam with a wavelength of 0.616 $\text{\AA}$  was used and collimated to a diameter of 15  $\mu\text{m}$  or 40  $\mu\text{m}$ . These analytical spots were precisely positioned by CCD camera, allowing the spatial distribution in coral microstructures to be directly investigated. The two-dimensional IP data were integrated and conducted to one dimensional intensity data.

Nano-textural observations and diffraction patterns of coral skeletons were performed by Transmission electron microscope (TEM) on JEOL JEM-2010 operated at 200kV. An ultra-thin section of the sample was prepared by finely polishing and Ar ion sputtering. No calcium oxide (CaO) in this analysis indicated that the original mineral phase had been maintained in the coral sample.

In order to verify the detection limit of strontianite in coral aragonite by using SR-XRD, we prepared the synthetic aragonite and strontianite minerals (Kanto chemical co., Inc).

The Sr-free aragonite powder was prepared by precipitation from aqueous solutions (modified from Lucas-Girot *et al.*, 2007). A solution of potassium hydrogen carbonate, 0.1 M, was prepared through the dissolution of  $\text{K}_2\text{CO}_3$  (Wako Pure Chemical Industries, Ltd.) in distilled water. A calcium chloride solution, 0.1M, was prepared by dissolving  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  (Kosa Chemical Co., Ltd.) in distilled water. The boiling solution of calcium chloride was added to the stirred boiling solution of potassium hydrogen carbonate. The precipitate was aged 5 min in the boiling mother solution and then filtered, washed with boiling water, and dried 110 $^\circ\text{C}$  over night. An XRD pattern showed that powder included aragonite and a little calcite.

We repeated the above cycle, but in the second cycle we added a small amount of aragonite powder, obtained in the first cycle, as seed crystals to the mother solution. The XRD pattern of this powder produced only aragonite peaks.

After the aragonite was synthesized, we intermixed the strontianite to the synthetic aragonite with 0, 0.117, 0.49, 1.26, 3.53, 5.25, 10.10 wt% respectively (see Table.1).

### Results and Discussions

#### Implication for Sr/Ca thermometry

Fig. 1 shows coral microstructures of *Porites lobata* observed by SEM and optical microscope. In this study, the sizes of the COC are about 5-30  $\mu\text{m}$  in diameter. Fig. 2 indicates one-dimensional intensity

Sr (wt %)	SrCO <sub>3</sub> (wt %)	CaCO <sub>3</sub> (mg)	SrCO <sub>3</sub> (mg)
5.99	10.1	20.07 $\pm$ 0.01	2.25 $\pm$ 0.01
3.12	5.25	23.42 $\pm$ 0.01	1.30 $\pm$ 0.01
2.10	3.53	104.29 $\pm$ 0.01	3.82 $\pm$ 0.01
0.75	1.26	105.38 $\pm$ 0.01	1.35 $\pm$ 0.01
0.29	0.487	106.26 $\pm$ 0.01	0.52 $\pm$ 0.01
0.07	0.117	109.18 $\pm$ 0.01	0.128 $\pm$ 0.01

Table.1: The amount of strontianite in the synthetic aragonite.

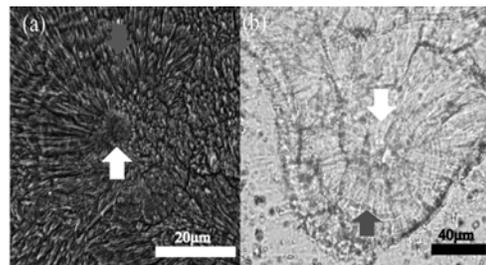


Figure.1: Coral skeletal microstructures of *Porites lobata* in scanning electron microscope (SEM) image (a) and petrographic thin section (b). The white arrow indicates centers of calcification (COC) and the gray arrow shows the emergent bundles of aragonite fibers surrounding the COC. Modified from Sowa *et al.* 2008.

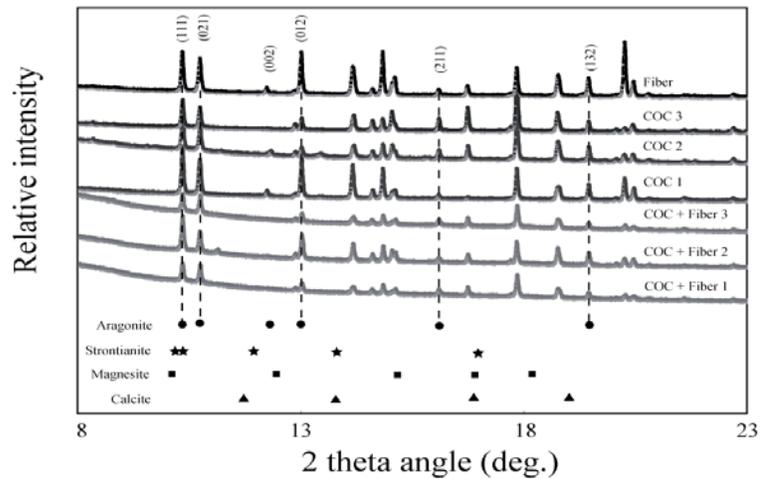


Figure.2: X-ray diffraction patterns of coral skeletal microstructures. Three of diffraction traces were analyzed by 40  $\mu\text{m}$  spot size in diameter (COC + Fiber1-3) and the others by 15  $\mu\text{m}$  (COC 1-3, Fiber). Aragonite, strontianite, magnesite and calcite are represented by solid circle (●), star (★), square (■), and triangle (▲), respectively.

patterns from 8 to 23 degrees 2-theta of the mineral phases in coral skeletal microstructures. The X-ray laser spot sizes of this study are 15  $\mu\text{m}$  and 40  $\mu\text{m}$  respectively, which correspond to the diffraction patterns of COC at three points, fiber of a single point, and an area that includes COC and fibers of three points. These diffraction patterns illustrate that COC and fibers consist only of aragonite.

To examine the mineral phase in coral skeletal nano-textures, we conducted TEM observations in COC and fibers. Coral skeletal nano-textures contain submicron, granular or needle-like shaped grains as shown in Fig. 3. The grain size of coral aragonite by our TEM observation resemble the nano-scale grain size observed by AFM (Cuif and Dauphine 2005). In the coral skeletal nano-textures, we did not find evidence for strontianite in the diffraction patterns of the coral skeletons (Fig. 3).

Fig. 4 illustrates the X-ray diffraction patterns of the six mixture samples of strontianite ( $\text{SrCO}_3$ ) and the synthetic aragonite ( $\text{CaCO}_3$ ) by SR-XRD. To detect the strontianite by qualitative analysis, we calculated and plotted the relative intensity of strontianite (111) normalized by aragonite (111). The regression line is  $Y = 2.83 \cdot X$  ( $R^2 = 0.96$ ), which was calculated from samples except 0.117 wt%. The relative intensity of 0.117 wt% was - 0.0673, which was under that of 0 wt%. This experimental result indicates that the lower limit of detection of strontianite in the synthetic aragonite is approximately 0.5 wt%, which corresponds to a Sr content of 2900 ppm (see Table 1). Greeger et al. (1997) reported that 3000 ppm (40 % of 7500 ppm) of Sr presented as strontianite. SR-XRD could detect the strontianite in coral skeletal microstructures if coral skeleton contains 3000 ppm of strontianite.

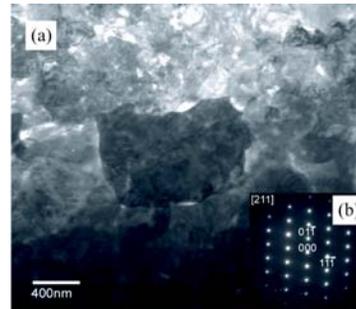


Figure.3: (a) Coral skeletal nano-textures observed by TEM. (b) Electron diffraction pattern of coral aragonite in a selected area.

In our study, however, with lower detection limits of approximately 0.5 wt% of strontianite and without any impurity phase conditions (Fig. 2), diffraction patterns in coral skeletal microstructures present only Bragg peaks at positions corresponding to the aragonite diffraction patterns. We could not detect strontianite and other mineral phase in the coral nano-textures from the diffraction patterns (Fig. 3). Our mineral phase analysis of coral skeletal microstructures and nano-textures are similar to the results of EXAFS and XANES, which showed no existence of strontianite or its intermediate state in coral skeletons (Finch *et al.*, 2003a, 2003b; Allison *et al.*, 2005).

Cohen et al. (2001) and Allison et al. (2005) reported that COC contain higher Sr/Ca ratio than fiber. However, there is no significant difference in the mineral phases of COCs and fibers in this study. These results imply that the Sr heterogeneity between COCs and fibers is not due to a difference in mineral phases. The causes of Sr heterogeneity are unclear and the mechanism of biological or kinetic effects remains to be resolved.

In summary, all diffraction patterns of coral skeletal

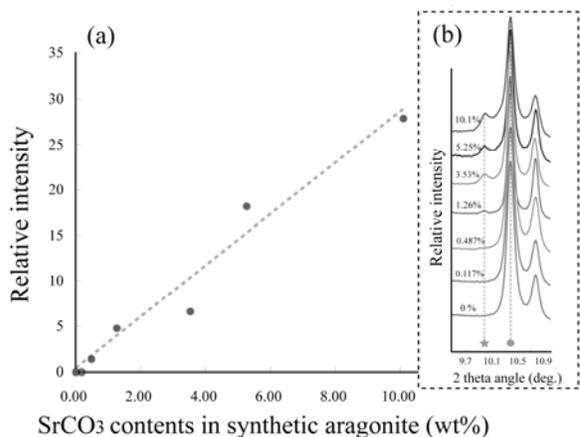


Figure 4: (a) Experimental detection limit of strontianite ( $\text{SrCO}_3$ ) by SR-XRD. Table.1 shows each contents of strontianite. Y-axis indicates that the relative intensity of strontianite (111) normalized by that of the synthetic aragonite (111). (b) The X-ray diffraction patterns of contents of strontianite against the synthetic aragonite; 0, 0.11, 0.49, 1.26, 3.53, 5.25 and 10.10 wt% respectively (see Table.1). Aragonite (111) and strontianite (111) indicate solid circle (●) and star (★) respectively.

microstructures and nano-textures (Figs. 3, 4) indicate that there is no evidence of strontianite in coral skeletons (within the lower limits of detection discussed above) and strontium is likely incorporated into the aragonite as solid solution.

The presence of strontianite remains possible, because our analysis focused only on limited areas in coral microstructures and nano-textures and we did not examine other coral species. Greegor et al. (1997) noted the presence of strontianite in *Montastrea annularis* and *Acropora palmata*. Here we investigated *Porites lobata* because it has been used as a paleo-environmental archive. It is possible that *Montastrea annularis* and *Acropora palmata* contain strontianite in their skeletons. Shirai et al. (2008) reported that Sr is heterogeneous in *Acropora nobilis* skeletal microstructures. Further investigation of the mineral phases of *Acropora* using SR-XRD may be useful.

Mineral phase analysis by SR-XRD and TEM corresponding to the coral skeletal microstructures and nano-textures show solely the aragonite diffraction pattern in this study. These results indicate that Sr/Ca thermometry of coral *Porites* should not consider the presence of strontianite in coral skeletons.

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