2012 2nd International Conference on Environment and BioScience
IPCBEE vol.44 (2012) © (2012) IACSIT Press, Singapore
DOI: 10.7763/IPCBEE. 2012. V44. 11

Biomineralization of Carbonate Minerals: Implications for Rhodolith Formation

Esther Roh [+] and Jiwon Sim

Hankuk Academy of Foreign Studies

Abstract. Rhodoliths play an important role in the production of carbonate sediments but it is still unclear how rhodoliths are formed. The objective of this study was to identify the formation factors and the mineralogical characteristics of rhodoliths. The microorganisms that were cultivated from the rhodoliths were inoculated into D-1 media with various ratios of Ca-:Mg-acetate(60:0~0:60 mM). XRD analyses showed that both precipitates and rhodoliths are Mg-rich calcite. Calcite was formed with Ca-:Mg-acetate(60:0, 60:20 mM) and Mg-rich calcite was formed with Ca-:Mg-acetate(30:30, 20:60 mM), which is close to the actual ratio of Ca:Mg in the seawater the rhodoliths were collected. Huntite was formed with Ca-:Mg-acetate(0:60 mM). Carbonate minerals were not formed without Ca, Mg-acetate. SEM-EDS analyses showed that Mg-rich calcite formed by the microorganisms had rhombohedron shape and consisted of Ca, Si and Mg with extracellular polymeric substance (EPS). These results indicate that the microorganisms induce precipitation of Mg-rich calcite on the cell walls and EPS via the accumulation of Ca and/or Mg ions on the cells. 16s rRNA analyses of the carbonate precipitating microorganisms show that they are *Proteus mirabilis*. Ca:Mg ratio and microorganisms control carbonate mineralogy, and microbial formation of carbonates may play an important role in Ca, Mg and C biogeochemistry as well as rhodolith formation and carbon fixation in natural environments.

Keywords: Rhodolith, Biomineralization, Carbonate minerals, Carbon fixation, Carbon cycle

1. Introduction

Rhodoliths are unattached calcareous, coralline red algae that form extensive beds (Konar et. al., 2006) widely distributed around the world. The formation of rhodoliths is known to be influenced by red algae (Woo, 2003). However, there is no proof that red algae influence the formation of the core of rhodolith. Therefore it can be assumed that rhodolith can be formed by other influences other than red algae. Considering the fact that rhodolith beds are only observed in several parts of the world, chemical processes of minerals in sea water can account for the formation of rhodoliths. Biological processes are another likely reason for the formation of rhodoliths since microbes have been known to participate in the formation of minerals. Thus we can conclude that rhodoliths may be formed by (1) red algae, (2) chemical saturation, or (3) microorganisms. The objectives of this study were to examine the mineralogical characteristics of the rhodoliths and to determine how they are formed.

The experiment will broaden our understanding of the key role in the formation of rhodoliths. Understanding the formation of rhodoliths and synthesizing rhodoliths to fix large amounts of CO_2 in the air will provide a large safe and permanent storage of CO_2, one of the main factors of global warming.

2. Materials and Methods

2.1. Source of Microorganisms

[+] Corresponding author. Tel.: + 82-10-3552-3202
E-mail address: rohesther@gmail.com.

In this experiment, the biological formation of rhodoliths was examined by using microorganisms enriched from sampled rhodoliths, reddish nodules with diameters of five to seven centimetres collected from the Western coast of Wu-Do, Jeju Island, South Korea. To enrich the microorganisms, sampled rhodoliths were crushed, added to autoclaved D-1 media, and cultivated in an aerobic condition with sunlight at 25°C for 7 days. The D-1 media (Table 1) is composed to provide similar conditions with the seawater of Wu-Do, Jeju Island to examine the actual possibility of biological formation of rhodoliths. The D-1 media was autoclaved at 121°C for 20 minutes to kill off any microorganisms that are unwanted. A week later, a new D-1 media, with the same compositions was made. 10 flasks were prepared with various concentrations of Ca-acetate and Mg-acetate: 0 mM, 30 mM, and 60 mM. Enriched microorganisms were inoculated to half of the flasks. Then the 10 flasks were cultured in an aerobic condition with sunlight at 25°C for 30 days. The enriched microorganisms were analysed by 16s rRNA gene DGGE analysis to determine the bacteria species. 16s rRNA gene analysis was carried out to observe the variety of species of microorganisms enriched from the sampled rhodoliths.

Table 1: Growth media (D-1) used for the enrichment of the carbonate forming microorganisms from rhodoliths

Components	*g/L*
Yeast extract	10
Proteose peptone	5
Glucose	1
NaCl	35

2.2. Growth Conditions

ICP-AES (Inductively Coupled Plasma Atomic Emission Spectroscopy) analysis showed that the ratio of Mg and Ca in the seawater near Wu-Do, Jeju Island, Korea is 3.09 to 1 (data not shown). In order to examine how different Ca and Mg ratios affect the biomineralization of carbonate minerals, various ratios of Ca and Mg-acetate were added to 100 mL of D-1 media, a total of five different groups: (1) 60 mM:0 mM, (2) 60 mM:20 mM, (3) 30 mM:30 mM, (4) 20 mM:60 mM, and (5) 0 mM:60 mM (Table 2). Since the actual Ca-to-Mg ratio in the sea water is 3 to 1, rhodoliths were expected to form the best in the ratio of 60 mM:20 mM.

Two 100 mL of D-1 media was prepared for each Ca-to-Mg-acetate ratio, a total of 10 flasks. These flasks were divided into two groups: Group 1 was NOT inoculated with the enriched and cultured microorganisms (control group) and Group 2 WAS inoculated with the enriched and cultured microorganisms. Both of groups were cultivated in an aerobic condition with sunlight at 25°C for around a month (38 days) to form carbonate minerals.

2.3. Analyses and Characterization

After one month, the precipitates that formed in the flasks were collected by centrifugation and desiccated in room temperature for 7 days. The dried precipitates were sent for X-ray Diffraction (XRD) analysis to determine the mineralogical composition of the precipitates. XRD analyses were performed by PANalytical X'Pert Multipurpose X-ray Diffractometer (The Analytical X-ray Company, Netherlands) equipped with Cu-Kα radiation with a scan rate of 5 °/min. The results were compared with the mineralogical composition of the sampled rhodoliths to see whether the precipitates formed were rhodoliths. Scanning Electron Microscope with Energy Dispersive X-ray Spectroscopy (SEM-EDS) was also used to analyze the shape and chemical composition of the precipitated carbonate minerals. SEM-EDS also determined the mineralogical characteristics of the rhodoliths.

3. Results and Discussion

3.1. Formation of Carbonate Mineral

Fig. 1 shows the experiment materials designed according to the five different Ca-to-Mg-acetate ratios. For each of the same Ca- and Mg-acetate ratio, one group was inoculated with the microorganisms enriched from the sampled rhodoliths, and the other was not (control group). The color of the inoculated group gradually turned milky as time passed. There were no changes found in the control group. After ten days, a small amount of precipitate formed in the inoculated group. Two weeks after the experiment setting, there were apparent differences in the color and turbidity between the inoculated group and the control group.

The amount of precipitates produced in the inoculated groups increased gradually during the month throughout the experiment. After one month, a lump of precipitate was observed in all five inoculated groups while the control groups exhibited no change. The control groups maintained their transparent colors. The precipitates produced in the inoculated groups were isolated from the media and washed with distilled water after centrifugation and was prepared for desiccation.

Table 2: Experimental design to examine the effect of different Ca, Mg ratios on biomineralization of carbonate minerals

Experiment	*Ca-acetate concentration (mM)*	*Mg-acetate concentration (mM)*	*Microorganism*
1	60	0	O*
	60	0	X
2	60	20	O
	60	20	X
3	30	20	O
	30	20	X
4	30	60	O
	30	60	X
5	0	60	O
	0	60	X

*O: with microorganism, X: without microorganism

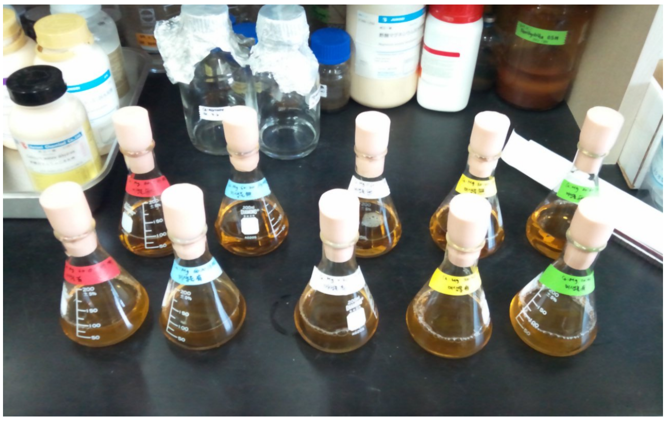

Fig. 1: Photo showing experimental setting for biomineralization of carbonate minerals by microorganisms enriched from rhodolith

3.2. Microbial Diversity

Fig. 2 shows the phylogenetic tree based on the 16s rRNA sequence analysis of the carbonate precipitating microorganism enriched from the sampled rhodoliths. The analysis showed that the enriched microorganisms included a carbonate forming microorganism *Proteus mirabilis*. According to the analysis, the precipitates formed in the experiments can be presumed to be carbonate minerals.

3.3. Mineralogical Characterization of Rhodoliths

XRD analyses of rhodoliths showed that d(104) = 2.99Å (calcite = 3.03Å, dolomite = 2.8Å) because Mg(ionic radius = 0.066 nm) substituted Ca(ionic radius = 0.099 nm) sites within calcite. From the result, the rhodoliths are identified as carbonate minerals, specifically Mg-rich calcite (Fig. 3). It is presumed from the results that greater concentrations of Mg than that of Ca in seawater cause Mg to replace Ca during the formation of rhodoliths. SEM-EDS analyses of the rhodoliths showed that the rhodoliths had a rhombohedron shape consisted of Ca and Mg.

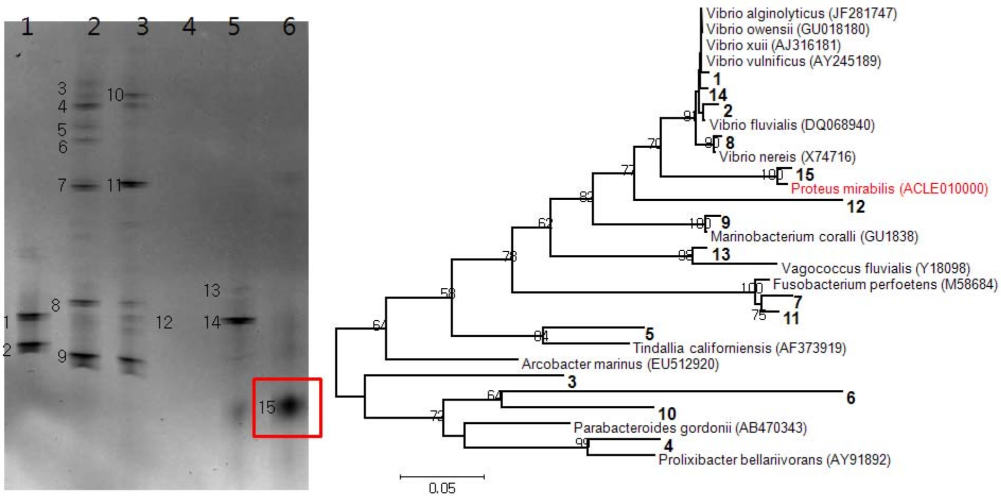

Fig. 2: DGGE patterns (left) and phylogenetic tree (right) of microorganism enriched from the rhodoliths

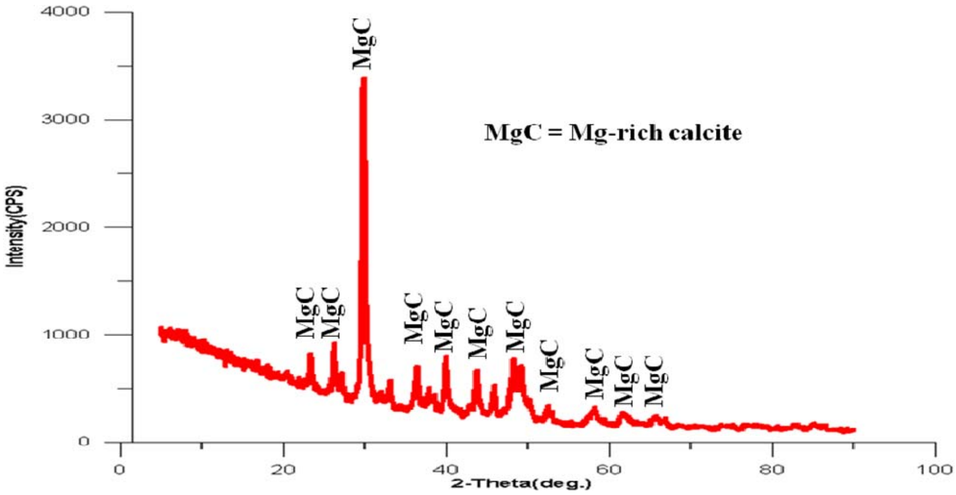

Fig. 3: XRD analysis of carbonate minerals precipitated by microorganism enriched from the rhodoliths

3.4. Mineralogical Characterization of Precipitated Carbonate Minerals

The microorganisms enriched from rhodoliths precipitated carbonate minerals in D-1 media containing different ratios of Ca- and Mg-acetate (Table 2). XRD analyses of the precipitates showed that the carbonate minerals were calcite, Mg-rich calcite, and huntite. For the media containing 60 mM:0 mM and 60 mM:20 mM ratios of Ca and Mg, XRD analysis of the precipitates produced showed that d(104) = 0.0302 nm and that the precipitates are mainly calcite ($CaCO_3$) (Fig. 4A and 4B). For the media containing 30 mM:30 mM ratio of Ca and Mg, XRD analyses showed that d(104) = 0.0299 nm (Fig. 4C). For the media containing 20 mM:60 mM ratio of Ca and Mg, XRD analyses showed that d(104) = 0.0299 nm (Fig. 4D). These two precipitates were identified to be Mg-rich calcite, like rhodoliths. Huntite($CaMg_3(CO_3)_4$) was precipitated in the 0 mM:60 mM ratio of Ca and Mg. XRD analyses showed that d(104) = 0.0284 nm (Fig. 4E).

SEM-EDS analyses showed that carbonate materials formed by various concentrations of Ca-, Mg-acetate had a rhombohedron shape. The carbonate minerals mainly consisted of Ca, C, and O when they were precipitated in 60 mM:0mM (Fig. 5A) and 60 mM:20 mM (Fig. 5B) ratios of Ca-, Mg-acetate. The carbonate minerals mainly consisted of Ca, Mg, and O when they were precipitated in 30 mM:30 mM (Fig. 5C), 20mM:60 mM (Fig. 5D), and 0 mM:60 mM (Fig. 5E) ratios of Ca-, Mg-acetate.

This study shows that rhodoliths is a type of carbonate mineral, Mg-rich carbonate. Mineral carbonates are one of the most permanent, safe, and largest ways to store CO_2 (Huijgen, 2004). Carbon dioxide (CO_2) is known to be a green house gas that contributes greatly to global warming, a threat that has been bringing various changes in the climate and sea water level. Sequestration of CO_2 is needed and according to this study, fixation of CO_2 occurs when rhodoliths are formed. The problem is that reaction rates and the formation of minerals take too long (Huijgen, 2004). In nature it takes an average of 500 years for rhodoliths to form (Littler et al., 1991). However, through the usage of the microorganism *Proteus mirabilis,* large amounts of carbonate can easily be synthesized and collected.

This study shows that rhodoliths are Mg-rich carbonate. Another research paper has studied how dolomite is formed and showed many similarities with this study throughout its results, methods, and materials (Sánchez-Román et al., 2011). The paper showed the formation of dolomite where the level of Mg was much higher than that of Ca. However, through various ratios of Ca and Mg, our study proved that the formation of rhodoliths also occurred when the level of Mg was much higher than that of Ca.

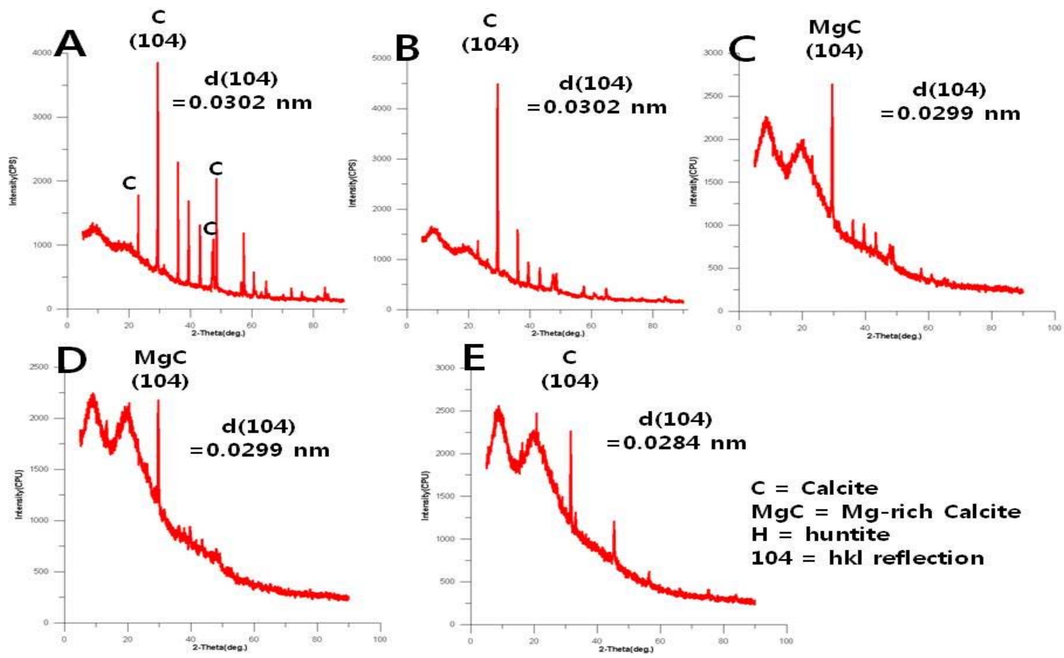

Fig. 4: XRD analyses of carbonate minerals precipitated by the microorganism using different ratios of Ca- and Mg-acetate

4. Conclusion

XRD and SEM-EDS analyses have shown that precipitated carbonate minerals are Mg-rich calcites with rhombohedran crystal shapes, the same mineral as rhodoliths collected from Wu-Do. The difference in ratios of Ca and Mg showed why rhodoliths were Mg-rich calcite and how Mg took the place of Ca site in the crystal structure of the calcite precipitated by the microorganism enriched from rhodoliths near Wu-Do. Thus, we can conclude that rhodoliths are formed by biological processes rather than chemical processes in natural environments.

Further research can be conducted on how much CO_2 can actually be stored in rhodoliths and how synthesized rhodoliths can be used for CO_2 fixation to prevent global climate change. Further research can also be conducted to identify indigenous microorganisms responsible for the formation of rhodoliths in different parts of the world, comparing and contrasting the structures of various rhodoliths.

5. Acknowledgements

The authors would like to thank Professor Yul Roh, of the Department of Earth and Environmental Sciences at Chonnam National University for his valuable comments and inspiration he extended. We also owe are deepest gratitude to Mr. D. M. Song and W. J. Moon in KBSI-Gwangju Branch for TEM/SEM-EDX analyses and Mr. C. Kim in EMMC of Chonnam National University for XRD analysis, making available the data.

6. References

[1] Huijgen, W.J.J., Comans, R.N.J., Witkamp, G.J. (2004). CO_2 sequestration by mineral carbonation. Poster presentation at the Nertherlands Process Technology Symposium (NPS4), Veldhoven, The Netherlands.

[2] Konar, B., Risomena-Rodriguez, R., Iken, K. (2006). Rhodolith bed: a newly discovered habitat in North Pacific Ocean. *Botanica Marina,* 49, 355–359.

[3] Littler, M.M., Littler, D.S., Hanisak, M.D. (1991). Deep-water rhodoliths distribution, productivity, and growth history at sites of formation and subsequent degradation. *J. Exp. Mar. Biol. Ecol.,* 150, 163-182.

[4] Sánchez-Román, M., Romanek, C.S., Fernández-Remolar, D.C., Sánchez-Navas, A., McKenzie, J.A., Pibernat, R.A., Vasconcelos, C. (2011) Aerobic biomineralization of Mg-rich carbonates: Implications for natural environments. *Chemical Geology,* 281, 143–150.

[5] Woo, K.S., Kim, J.K., Kang, S.S. (2003) Characteristics and Formation conditions of the Rhodoliths in Wu Island beach, Jeju-do, Korea: Preliminary Report. *Journal of the Korean Society of Oceanography,* 8(4), 401-410.