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Bacterial degradability of dissolved organic carbon in coral mucus

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Abstract
Bacterial decomposition of dissolved organic carbon (DOC) in coral mucus was investigated under dark conditions for 7 months to reveal what percentage of the coral-derived DOC is rapidly utilized by bacteria, and conversely, what percentage remained for a long term as refractory organic matter. During the first week, 38%-47% of DOC released from the hermatypic corals *Acropora pulchra* and *Porites cylindrica* were mineralized by bacteria collected from natural reef water. The bacterial abundance dramatically increased from the order of 10^3 to 10^6 cells ml^{-1} during the 1 week. Some part of the remaining organic C at 1 week was slowly decomposed over 3 months, but further degradation was not observed thereafter. Finally, 32%-44% of the initial DOC was not mineralized over 7 months. These results suggest that, under dark conditions, DOC in coral mucus is not completely degraded by free-living bacteria and contributes to long-term C fixation as refractory organic matter.

Keywords: coral, mucus, bacteria, mineralization, refractory organic matter

Introduction

It is well known that coral colonies release organic matter to the ambient seawater as dissolved and particulate organic matter (DOM and POM, respectively) (Tanaka et al. 2008). The coral-derived organic matter is often collectively referred to as mucus. The coral mucus has been regarded as ecologically important: bacterial aggregation was found in coral mucus (Ducklow and Mitchell 1979) and coral exudates actually enhanced the growth of pico- and nanoplankton (Ferrier-Pagès et al. 2000). Wild et al. (2004a, b) showed that a part of coral mucus was rapidly mineralized into CO_2 by bacteria in the reef sediment. The organic matter released from corals has been considered to be a good source of energy for microorganisms and to be completely and rapidly mineralized into CO_2 in the coral reef ecosystem.

However, some studies proposed negative ideas on the bacterial decomposition of coral mucus. Vacelet and Thomassin (1991) observed that coral mucus of high molecular weight (more than 6000−8000) was not completely degraded by bacteria and eukaryotes even after 21 d. Krupp (1984) and Coffroth (1990) concluded that coral mucus itself is a material of low nutritional value for reef organisms and that the property to collect organic detritus and to serve as sites of aggregation for microorganisms is a more important trophic role of coral mucus. More recently, Ritchie (2006) and Chen et al. (2007) found antibacterial substance from coral mucus. These studies suggest that some part of the organic matter released from corals is not rapidly mineralized by bacteria but remain as relatively refractory organic matter.

Therefore, no previous studies have revealed what % of the coral-devied organic matter is rapidly decomposed by bacteria and what % is not. The purpose of this study is to quantitatively investigate bacterial degradability of coral mucus for a long term. Dissolved organic matter in pure coral mucus was put under dark and the change in organic C concentration due to bacterial mineralization was observed over 7 months.

Material and methods

The experiment was performed using two hermatypic corals *Acropora pulchra* and *Porites cylindrica*, which were collected on the reef crest of Shiraho reef in Ishigaki Island (24° 21′-31′ N, 124° 4′-16′ E), Japan in Jul 2007. In Shiraho reef, the reef crest is developed roughly parallel to the shoreline at a distance of ca. 850 m. The corals were collected ca. 700 m offshore, where the depth was ca. 0.5 m at low tide. Six coral branches were cut from several colonies, and put in two glass bottles (i.e., three branches per bottle) for each coral species (total four bottles). The glass bottles (900 ml) contained 300 ml of artificial seawater (ASW). Surface area of each coral branch was 24-53 cm^2 (average 41 cm^2). The water volume was enough to cover the corals. To minimize organic matter contamination in the prepared ASW,

sodium chloride (NaCl), magnesium sulfate ($MgSO_4$), potassium chloride (KCl), and calcium chloride ($CaCl_2$) were combusted at 450˚C for 3 h in advance.

After the collection of the coral branches, the glass bottles were immediately carried to the laboratory. During the transportation, the corals produced much mucus due to the stress of gentle water turbulence in the bottles. Temperature of the seawater in the bottles was same as that of in situ reef water (29-30˚C). In the laboratory, the coral branches were taken out of the bottles. The duration of mucus production was 1 h. 400 ml of ASW was added to each bottle to dilute the produced mucus, and 5 ml of in situ reef seawater was also added to inoculate bacteria in the water column. The sample seawater (total 705 ml for each bottle) was then filtered with pre-combusted Whatman GF/F filters (pore size: 0.7 μm) to remove particulate organic matter (POM) including flocculated coral mucus and expelled zooxanthellae. The filtrate was put in pre-combusted glass bottles (1 L), and the bottles were placed under dark to observe mineralization of DOM in the filtrate. To make same temperature condition, the dark room was kept at 20˚C.

Sampling of the filtrate was conducted at 0, 2, 7, 30, 90, and 220 days of the dark incubation. 15 ml of the seawater was taken into pre-combusted glass ampoules to measure the concentrations of total organic C (TOC) and inorganic nutrients. The ampoules were immediately sealed and stored at -20˚C until analysis. 10 ml of the seawater was taken into acrylic tubes and fixed with pre-filtered formalin (final concentration 2%, v/v) to measure bacterial abundance.

The concentration of TOC was measured by the high temperature catalytic oxidation method (HTCO) using TOC-5000 (Shimadzu). Milli-Q water was used for blank and coefficients of variance for the analysis were <5%. Dissolved inorganic N (DIN: NO_3^-, NO_2^-, NH_4^+) and PO_4^{3-} concentrations were quantified with a nutrient analyzer AACS-III (BRAN+LUEBBE). Analytical error of the nutrient measurement was <0.02 μmol l^{-1}. Duplicate samples from each bottle were analyzed for TOC and inorganic nutrients.

The abundance of bacteria fixed by formalin was counted with an epifluorescence microscopy after staining with SYBR Gold. Bacterial aggregates were disintegrated by weak ultrasonic treatment for 20-30 s. At least 400 bacterial cells were counted from 20-25 fields, except for the samples at t = 0 d. For the samples at t = 0 d, 80-170 cells were counted from >40 fields, due to the small bacterial abundance.

The production rate of DOM (R: μmol cm^{-2} h^{-1}) by the corals was calculated from the organic matter (C: μmol l^{-1}) accumulated in seawater.

$$R = C\,V\,S^{-1}\,T^{-1} \quad (1)$$

where V, S and T are seawater volume (l), coral surface area (cm^2), and the duration of mucus production (h), respectively. Coral surface area was determined by aluminum-foil method (Marsh 1970). ASW used for the experiment initially had minor contamination of organic C (4.8 μmol l^{-1}), compared to the DOC produced by the corals (see below), thus, the C amount was subtracted from all the measured concentrations of TOC at each sampling time, supposing that the initially-contained organic C did not change during the experiment.

Bacterial growth efficiency (E) during the dark incubation was calculated as follows.

$$E = \Delta BC\,(\Delta TOC)^{-1} \quad (2)$$

ΔBC and ΔTOC are an increased bacterial C (μmol l^{-1}) and a decreased TOC (μmol l^{-1}) during a period, respectively. Bacterial C was estimated from determined bacterial number using the conversion factor of 15-30 fg C per bacterial cell (Fukuda et al. 1998).

Results and discussion

DOC accumulated in ASW by the range of 80-97 μmol l^{-1} (Table 1). The release rates normalized to the coral surface area were 400-440 nmol cm^{-2} h^{-1} for *A. pulchra* and 510-650 nmol cm^{-2} h^{-1} for *P. cylindrica* (range of duplicate bottle incubations) (Table 1). The release rates were comparable to those of *Acropora millepora* under the stress of air

Table 1 Initial conditions of the decomposition experiment. 'Conc.' means DOC concentration accumulated in the seawater. Release rates are normalized to the coral surface area. DIN includes nitrate, nitrite, and ammonium. Error bars indicate standard error of analysis

	DOC		DIN	PO_4^{3-}	Bacteria
	Conc. (μmol L^{-1})	Release rates (nmol cm^{-2} h^{-1})	(μmol L^{-1})	(μmol L^{-1})	(10^3 cells ml^{-1})
Acropora 1	80	444	0.86	0.24	4.4±3.0
Acropora 2	84	399	0.92	0.23	2.9±1.5
Porites 1	85	514	1.0	0.19	3.9±3.0
Porites 2	97	650	1.2	0.19	7.8±6.3

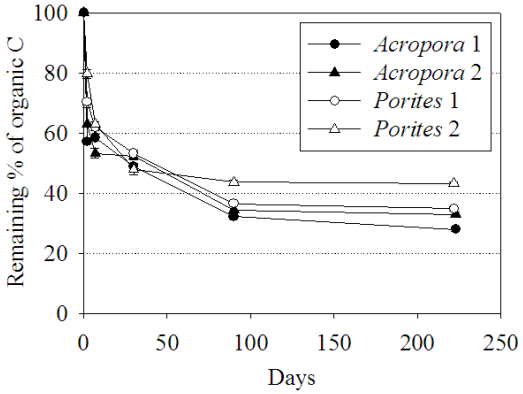

Fig. 1 Bacterial mineralization of dissolved organic carbon released from the four corals. Data are shown as remaining % of the initial concentrations, which ranged from 80-97 μmol l^{-1}. Error bars indicate standard deviation of analysis.

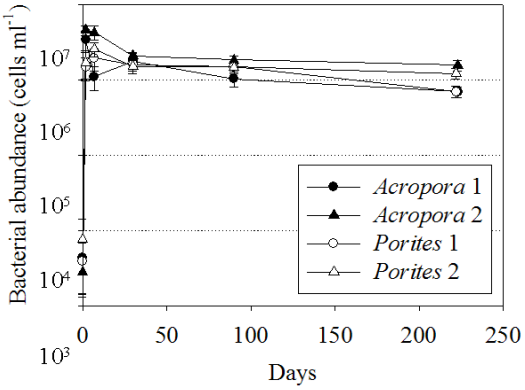

Fig. 2 Bacterial abundance during the organic matter decomposition. Data are indicated as mean ± standard error of analysis.

exposure (980±660 nmol cm^{-2} h^{-1}; Wild et al. 2005). Using the same coral species as the present study, Tanaka et al. (2008) measured DOM and POM release rates from the submerged corals as naturally as possible. The observed DOC release rates were 380 and 340 nmol cm^{-2} d^{-1} for *A. pulchra* and *P. cylindrica*, respectively (Tanaka et al. 2008). Assuming that DOC was continuously released in natural condition at daytime, the release rates of 380 and 340 nmol cm^{-2} d^{-1} would be one order of magnitude lower than those observed in the present study (400-650 nmol cm^{-2} h^{-1}). Wild et al. (2005) also compared the release rates of particulate organic C from *A. millepora* between the corals in air exposure and in a submerged condition, and showed that the release rates under the stress (980±660 nmol cm^{-2} h^{-1}) were about 10 times higher than those in the submerged.

This is the first study which observed the mineralization of DOC released from corals in the time scale of several months. The concentration of TOC dramatically decreased by the range of 32-39 μmol l^{-1} during the first 1 week (Fig. 1). Decreased % was very similar among the four incubations (37%-41%; Table 2). As the organic C was decomposed, NH_4^+ concentration increased from 0.8-0.9 μmol l^{-1} to 5.1-6.1 μmol l^{-1} during the 1 week, indicating that DON released from the corals was simultaneously mineralized. Bacterial abundance increased within the first 2 d by a factor of 1000, i.e. from 10^3 to 10^6 cells ml^{-1} (Fig. 2). Bacterial growth efficiency during the 2 d was calculated to be 12%-37% for *A. pulchra* and 7%-21% for *P. cylindrica*. These are usual values for the growth efficiency of marine bacteria in log phase (4%-30%; Carlson and Ducklow 1996), suggesting that labile organic matter in the coral mucus had similar degradability to that produced in other marine ecosystems, mainly by phytoplankton.

After 1 week, the concentration of TOC slowly decreased until Day 90 (Fig. 1). Average remaining % after 90 d was 37% (Table 2). First order decay constants (*k*) during 7-90 d were 0.004-0.007 d^{-1}, which were one order of magnitude lower than those of the first 1 week (0.066-0.077 d^{-1}; Table 2). After 90 d, TOC concentration decreased only by 1%-4% during the next 130 d (Fig. 1). Average remaining % of organic C after 220 d was 35% (Table 2). *k* values during 90-220 d were 0.0001-0.001 d^{-1} (Table 2). Considering that the decay constants were distinctly different among the three phases (i.e., 0-7 d, 7-90 d, and 90-220 d), the change in TOC concentration in Fig. 1 was not dependent on the concentration but on the different degradability of the organic matter during each phase. It can be defined that the organic matter decomposed during 0-7 d, 7-90 d, and 90-220 d is relatively labile fraction (F_L), semi-labile fraction (F_S), and refractory fraction (F_R), respectively. Supposing that the three defined fractions (F_L, F_S, and F_R) were only mineralized during each period (i.e., 0-7

Table 2 Remaining % of organic C at each sampling time. *k* values are first order decay constants during each decomposition phase.

	Period (d)	Remaining %	k (d^{-1})
Acropora 1	0-7	100 → 59	0.077
	7-90	59 → 32	0.007
	90-223	32 → 28	0.001
Acropora 2	0-7	100 → 53	0.090
	7-90	53 → 35	0.005
	90-223	35 → 33	0.0004
Porites 1	0-7	100 → 62	0.069
	7-90	62 → 37	0.006
	90-222	37 → 35	0.0003
Porites 2	0-7	100 → 63	0.066
	7-90	63 → 44	0.004
	90-222	44 → 43	0.0001

d, 7-90 d, 90-220 d), the percentage of each fraction can be calculated from the decreased % in Table 2: F_L, F_S, and F_R accounted for 37%-47%, 19%-26%, and 32%-44%, respectively (on average, F_L: 41%, F_S: 22%, F_R: 37%). Adding up F_L and F_S, about 60% could be mineralized by the bacteria within 3 months.

The present study has quantitatively shown that a part of the organic matter released from the corals is not mineralized into CO_2 by free-living bacteria in the reef water. There existed some inorganic nutrients in the incubated seawater at the end of the experiment (t = 220 d): 7.8-10.2 μmol N l^{-1} and 0.38-0.55 μmol P l^{-1}. Thus, bacterial incorporation of the remaining organic C could not be limited by the availability of N and P. Some possibilities are suggested for the remaining organic C. First, the organic matter released was originally recalcitrant to bacterial decomposition. Using coral mucus with molecular weight larger than 6000−8000, it was observed that the mucus web was not completely degraded by bacteria and eukaryotes even after 21 d (Vacelet and Thomassin 1991). They explained that mucus excretion from coral is a defensive reaction against physical and chemical stresses, thus mucus is a poor, or even inhibiting medium for the bacterial degraders isolated from the mucus itself. Similar results were also reported by Pascal and Vacelet (1981). Krupp (1984) and Coffroth (1990) also suggested that coral mucus is a material of low nutritional value for reef organisms. More recently, Ritchie (2006) observed that mucus from *Acropora palmata* had antibiotic properties that were likely to play a role in ordering only beneficial microbial communities on the coral surface. Antibacterial substance was also found from coral mucus of *Symphyllia gigantea* (Chen et al. 2007) and from tissue extraction of some soft corals (Kelman et al. 2006). Coral colonies might routinely produce organic matter resistant to the degradation by bacterial communities in the water column.

The second possibility explaining the remaining organic matter is that coral-derived organic matter was once utilized by bacteria and converted into different organic forms, which were recalcitrant to further bacterial decomposition. Ogawa et al. (2001) reported that when marine bacteria utilized labile compounds (glucose, glutamate), they produced refractory DOM (R-DOM) that persisted for more than a year. Their result suggested that microbial processes alter the molecular structure of DOM, making it resistant to further degradation. Considering that 5%−7% of original substrate C (glucose, glutamate) were altered to R-DOM in Ogawa et al. (2001), R-DOM produced by bacteria in the present study could account for only a minor part of the remaining organic matter (Fig. 1 & Table 2), supposing that the bacteria produced R-DOM with the similar ratio. Therefore, the preservation of the organic matter in the present experiment (ca. 35%) could not be explained only by bacterial activity to produce R-DOM, but some parts of the remaining organic matter could originally have resistance against bacterial decomposition.

Wild et al. (2004a, b) pointed out the important role of bacteria in reef sediment to decompose coral mucus. They considered that a highly diverse and dense sedimentary bacterial population, adapted to the decomposition of more refractory material, can decompose coral mucus more effectively than the bacterial community in water column. Because only the bacteria in water column were inoculated in the present study, the possibility that sedimentary bacteria decompose the remaining organic matter needs to be investigated in the future. However, a part of coral-derived organic matter could diffuse and be exported to the outer ocean without settling down to the sediment, especially in the reef where water residence time is short. For example, in the study site of the present study (Shiraho reef), average residence time of the lagoon water was estimated to be 4-8 h (H. Yamano unpubl. data). Hata et al. (2002) concluded that 6%-7% of gross primary production in Shiraho reef was exported to the outer ocean as POC. Their study also suggested the export of DOC from the lagoon. The present paper showed that considerable parts of the exported DOC might remain as refractory organic matter over several months.

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