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Bacterial production is enhanced by coral mucus in reef systems

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ABSTRACT

Coral mucus influences the abundance and composition of microbes in the surrounding seawater. Here, the hypothesis that the mucus of the coral genera *Acropora*, *Favia*, *Favites*, and *Goniastrea* would influence bacterial growth (production) in seawater (measured using ^{3}H-thymidine and ^{3}H-leucine incorporation methods) was tested. The bacterial production in mucus-supplemented seawater was significantly greater than that in unsupplemented seawater: production in the mucus-supplemented seawater ranged from 108.2 to 4111 μg C·L^{-1}·d^{-1} when measured by thymidine incorporation and from 72.9 to 115.9 μg C·L^{-1}·d^{-1} when measured by leucine incorporation. This study showed that the enhanced production may be due to bacteria originating from seawater and not from coral mucus. Nonetheless, the effect of mucus on bacterial production was different among the coral genera: the mucus of *Favia* did not always enhance bacterial production. Additionally, even a very small amount of coral mucus, only ~300 μL of mucus per liter of seawater, was sufficient to enhance bacterial production by approximately 2-fold compared to unsupplemented seawater. These results suggest that coral mucus has a significant impact on the marine biogeochemical cycle around coral reefs.

1. Introduction

Corals release mucus into the surrounding environment. There are several functions of coral mucus: it acts primarily as a defense mechanism against various stressors such as pollution, ultraviolet light damage, desiccation, and calcification (Brown and Bythell, 2005). Coral mucus plays an important role in preventing coral disease (Krediet et al., 2013). Richie (2006) showed that the mucus from *Acropora palmata* inhibits bacterial growth (production) in seawater by up to 10-fold. Additionally, that report indicated that 20% of the cultured bacteria from the mucus of *A. palmata* had an antibiotic activity against tester strains, including a coral pathogen that causes white pox disease. Wild et al. (2004) reported that coral mucus works as an energy carrier and a particle trap in the reef system, suggesting that mucus reduces losses of energy and nutrients from the reef system to the outside. Many previous studies reported that compared to seawater, coral mucus contains higher concentrations of organic matter (e.g., Tanaka et al., 2011; Wild et al., 2004), nutrients (e.g., Nakajima et al., 2009; Wild et al., 2005), and microbes such as viruses, bacteria, archaea, and protozoa (e.g., Rohwer et al., 2002; Wegley et al., 2007). Tanaka et al. (2011) showed that the concentrations of total organic carbon and nitrogen in coral mucus are 2–4 times higher than those in the surrounding seawater. Bacterial abundance in coral mucus is also 3–6 times higher than that in the surrounding seawater (Garren and Azam, 2010). In light of these characteristics of the coral mucus, the effect of coral mucus released into the surrounding water on ambient microbes should not be ignored (Bythell and Wild, 2011).

Coral mucus influences abundance and composition of microbes in the surrounding seawater. Previous reports showed a remarkable increase in microbial abundance in seawater supplemented with coral mucus (e.g., Herndl and Velimirov, 1986; Nakajima et al., 2009). Ferrier-Pages et al. (2000) showed that the cell abundance and carbon biomass of pico- and nanoplankton are increased approximately 6-fold by mixed mucus of four or five corals in mesocosms. Allers et al. (2008) assessed the abundance and bacterial taxa that are stimulated by mucus of *Fungia* spp. They showed an approximately 5-fold increase in bacterial abundance and a change in bacterial community structure; after stimulation with the mucus, there was a steep rise in the number of Gammaproteobacteria, particularly Alteromonadaceae, followed by Vibrionaceae. Although almost all of the reports have a methodological problem—they equate the increase of the bacterial abundance with bacterial production—these data mean that coral mucus should have a great impact on the biogeochemical cycle via microbes in a reef ecosystem.

In this study, bacterial production in seawater supplemented with coral mucus was measured by means of the radioisotope technique: thymidine and leucine incorporation methods. Bacterial production estimated using measurement of the increase in bacterial abundance strictly speaking is not accurate because abundance is the result of both growth and mortality. The radioisotope technique allows us to

Abbreviations: SW, seawater; MuS, seawater supplemented with coral mucus; MuFS, 0.2-μm-filtered seawater supplemented with coral mucus.

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measure bacterial production more accurately and precisely. Moriarty et al. (1985) measured spatiotemporal variation in bacterial production in a water column over a coral reef using the thymidine method. They showed relatively higher bacterial production levels in the water column within a coral reef compared to the reef front and open water. Nakajima et al. (2013) also showed that the high abundance of bacteria, cyanobacteria, and nanoflagellates and strong bacterial production in the sea surface microlayer over coral reefs are probably due to coral mucus. These reports imply that the coral mucus might be a driving force behind the microbial life cycle in a reef system.

The hypotheses tested in the present study are as follows: (i) growth of the bacterial fraction originating from the seawater is enhanced by the mucus released, (ii) the effect of the coral mucus on bacterial production differs among coral genera, and (iii) even very small amounts of mucus can influence bacterial production. To this end, 3 types of experimental treatment were utilized: seawater only (SW), seawater supplemented with coral mucus (MuS), and 0.2-μm-filtered seawater supplemented with coral mucus (MuFS). Four coral genera were selected that are predominant in this study area: *Acropora*, *Favia*, *Favites*, and *Goniastrea*. When production in MuS was higher than that obtained with SW and MuFS, it was assumed that bacterial growth in seawater is enhanced by coral mucus. Conversely, when the production in MuS was lower, it was assumed that the bacterial growth in seawater is suppressed by coral mucus.

2. Materials and methods

2.1. Sample collection

Seawater and corals were collected near Kushimoto (33°29′12.1″N, 135°41′29.3″E), Wakayama Prefecture, Japan, in October 2011 and April and July 2012. Environmental parameters, water temperature, salinity, chlorophyll *a* concentration, and turbidity at 1 m depth were measured using a modernized compact and light-weight multiparameter water quality meter AAQ1183-H (JFE Advantec Co., Ltd., Hyogo, Japan; Table 1). Seawater was collected at a site distant from corals. One to three colonies of corals *Acropora* sp., *Favia* sp., *Favites* sp., and *Goniastrea* sp., which are dominant species in that area, were collected. Coral mucus was obtained directly by means of air exposure of the corals for 10 min after washing with 0.22-μm-filtered natural seawater to remove bacteria from the coral surface (Tanaka et al., 2011; Wild et al., 2004). After the mucus was collected, further analyses were conducted within 1 h.

2.2. Incorporation of thymidine and leucine

To measure bacterial production, 3 growth conditions for each coral were set up in 3 autoclaved polycarbonate bottles washed with 1 N HCl and Milli-Q water: seawater (SW), seawater supplemented with the coral mucus (MuS), and 0.2-μm-filtered seawater supplemented with the mucus (MuFS) at a mucus:seawater ratio of 1:300 (v:v). After gentle mixing, the bottles were incubated for 30 min and then subjected to radioisotope analyses for measurement of bacterial production. This measure was quantified by means of incorporation of ^{3}H-thymidine or ^{3}H-leucine according to the procedures described previously (Fuhrman and Azam, 1982; Kirchman, 2001). Briefly, triplicate 5-mL samples and one control sample killed with 5% trichloroacetic acid (TCA) were incubated for 1–5 h with 10 nM ^{3}H-thymidine (63–83.2 Ci mmol^{-1}; MT-6039; Moravek Biochemicals, Inc., Brea, CA, USA) or 20 nM ^{3}H-leucine (54.1–165.2 Ci mmol^{-1}; NET135H; PerkinElmer, Inc., Waltham, MA, USA) at the in situ temperature. After extraction of macromolecules with ice-cold TCA followed by filtration, the filters were radioassayed by means of a liquid scintillation counter using the Filter Count scintillation cocktail (PerkinElmer Inc., Waltham, MA, USA). In July 2012, the effect of dilution of coral mucus on bacterial production was estimated using thymidine incorporation. Corals used in this dilution experiment were *Acropora* and *Favia*. In addition to the dilution ratio of mucus:seawater at 1:300, the ratios of 1:30 and 1:3000 were prepared. In the thymidine incorporation experiment, bacterial abundance was converted to carbon mass using the conversion factor, 30.2 fg C·cell^{-1} (Fukuda et al., 1998). In the leucine incorporation experiments, bacterial abundance was converted to carbon units according to the method of Kirchman (2001). Cell-specific production was calculated by dividing the bulk production based on thymidine and leucine incorporation by bacterial abundance. Statistical differences in the bacterial production rates were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's honest-significant-difference (HSD) test.

Table 1
Environmental characteristics in surface seawater.

Sampling date	WT (°C)	Salinity	Chl *a* (μg/L)	Turbidity (FTU)
October 2011	24.01	34.08	0.26	0.21
April 2012	18.72	34.57	0.49	0.53
July 2012	25.64	32.58	0.41	0.70

* WT: water temperature, Chl: chlorophyll, FTU: formazin turbidity unit.

2.3. Estimation of bacterial abundance

All samples were fixed with 0.22-μm-filtered paraformaldehyde (final concentration, 2% w/v). The fixed sample was filtered onto a polycarbonate membrane filter (0.2 μm pore size) under vacuum (<100 mm Hg), and stained with a 2 μg mL^{-1} solution of 4′,6-diamidino-2-phenylindole (DAPI) (Porter and Feig, 1980). More than 300 cells per sample or at least 10 microscopic fields were counted under an epifluorescence microscope (BX51; Olympus Corporation, Tokyo, Japan; filter used was U-MWU2). Statistical differences in the bacterial production rates were evaluated using one-way ANOVA followed by Tukey's HSD test.

3. Results

3.1. Bacterial abundance

In the SW samples, bacterial abundance ranged from 5.2×10^5 to 8.3×10^5 cells mL^{-1} (Table 2). Abundance in the MuS samples ranged from 7.0×10^5 to 1.1×10^6 cells mL^{-1} and was not significantly different among coral genera. In MuFS samples, the abundance in October 2011 was 1 order of magnitude higher compared to the other groups: the abundance was in the range of 1.3×10^4 to 2.8×10^5 cells mL^{-1}. Taking into account the mucus dilution ratio of 1:300, bacterial abundance in coral mucus was more than 10-fold higher than that in seawater, ranging from 4.0×10^6 to 8.4×10^7 cells mL^{-1} (data not shown).

3.2. Bacterial production as assessed using thymidine and leucine incorporation

Bacterial production ranged from 68.9 to 123.3 μg C·L^{-1}·d^{-1} according to thymidine incorporation in the SW samples; values of 39.9 and 86.0 μg C·L^{-1}·d^{-1} were obtained according to leucine incorporation (Table 2). For 3 of 4 coral genera (*Acropora*, *Favites*, and *Goniastrea*) bacterial production estimated using thymidine incorporation in the MuS samples was significantly higher than that in the SW samples, ranging from 108.2 to 4111 μg C·L^{-1}·d^{-1}. When measured by leucine incorporation, bacterial production in the MuS samples of *Acropora* and *Favites* also showed a tendency similar to the one observed with thymidine incorporation, ranging from 72.9 to 115.9 μg C·L^{-1}·d^{-1}. The trend in *Favia* was different from that in the other 3 species: bacterial production estimated using incorporation of thymidine and leucine in the MuS samples was not always higher but was instead lower than that in the SW samples (Table 2). In the MuFS samples, almost all bacterial production levels were significantly lower than those in the SW and