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Cultured fungal associates from the deep-sea coral *Lophelia pertusa*

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ABSTRACT

The cold-water coral *Lophelia pertusa* provides important habitat to many deep-sea fishes and invertebrates. Studies of the microbial taxa associated with *L. pertusa* thus far have focused on bacteria, neglecting the microeukaryotic members. This is the first study to culture fungi from living *L. pertusa* and to investigate carbon source utilization by the fungal associates. Twenty-seven fungal isolates from seven families, including both filamentous and yeast morphotypes, were cultured from healthy *L. pertusa* colonies collected from the northern Gulf of Mexico, the West Florida Slope, and the western Atlantic Ocean off the Florida coast. Isolates from different sites were phylogenetically closely related, indicating these genera are widely distributed in association with *L. pertusa*. Biolog^{TM} Filamentous Fungi microtiter plates were employed to determine the functional capacity of a subset of isolates to grow on varied carbon sources. While four of the isolates exhibited no growth on any provided carbon source, the rest ($n=10$) grew on 8.3–66.7% of carbon sources available. Carbohydrates, carboxylic acids, and amino acids were the most commonly metabolized carbon sources, with overlap between the carbon sources used and amino acids found in *L. pertusa* mucus. This study represents the first attempt to characterize a microeukaryotic group associated with *L. pertusa*. However, the functional role of fungi within the coral holobiont remains unclear.

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1. Introduction

Lophelia pertusa is one of the few cold-water coral species capable of forming large reef structures in the deep sea (Lumsden et al., 2007), acting as biodiversity hotspots that host many species of invertebrates and fishes (Reed, 2002). *L. pertusa* itself is host to a microbial community that is distinct from surrounding seawater and sediments (Schöttner et al., 2009; Yakimov et al., 2006). Corals, both cold-water and tropical, have been hypothesized to be part of a larger symbiotic association, termed the coral holobiont, that consists of the coral host and all associated microbiota including bacteria, archaea, and microeukaryotes (Wegley et al., 2004). In addition to gaining a general understanding of the roles of microbes in carbon cycling in deep benthic environments, determining the functional roles of different classes of microorganisms is of increasing importance as coral health declines worldwide (Rosenberg et al., 2007). Microbial communities associated with healthy and diseased corals differ drastically (Bourne et al., 2011; Pantos et al., 2003), offering evidence of the important roles that microbes play in coral health. Culture-independent methods are most often used to describe bacteria and archaea associated with corals (e.g., Bourne and Munn, 2005; Kellogg, 2004; Kellogg et al., 2009; Rohwer et al., 2001; Wegley et al., 2004); however, general 18S rRNA gene primers intended to survey the diversity of microeukaryotes (e.g., fungi and protists) can be overwhelmed by coral genetic material in DNA extractions. Culturing coral-associated microbiota with selective media offers an alternative to culture-independent community analysis, allowing additional biochemical, morphological and physiological characterization of isolates. However, as with bacteria, the diversity of the cultured fungal isolates may be a gross underrepresentation of the true phylogenetic diversity found in situ.

Fungi are known to associate with shallow-water corals, both as potential symbionts (Bentis et al., 2000; Domart-Coulon et al., 2004; Ravindran et al., 2001) and pathogens (Raghukumar and Raghukumar, 1991; Yarden et al., 2007; Zuluaga-Montero et al., 2010). One of the major diseases of sea fans (*Gorgonia* spp.) is caused by the ascomycete *Aspergillus sydowii* (Geiser et al., 1998; Smith et al., 1996). Most of the fungi described in shallow-water corals are endolithic, occurring primarily in the underlying carbonate skeleton and not affecting the coral tissue (Bentis et al., 2000; Campion-Alsumard et al., 1995a, 1995b). The function of fungi associated with corals is not well understood. A metagenomic study by Wegley et al. (2007) showed that fungal

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metabolic genes in the shallow-water coral *Porites astreoides* were dominated by nitrogen cycling (e.g., nitrate/nitrite ammonification, ammonia assimilation), which would be potentially useful for corals in nitrogen-limited waters. A similar study by Vega Thurber et al. (2009) showed that the majority of fungal genes associated with *Porites compressa* were similar to phytopathogenic fungi, and more of the phytopathogenic-like fungal genes were found when stressors were applied to the coral.

Fungi have been cultured from a variety of deep-sea locations, including hydrothermal vent fauna (Burgaud et al., 2010, 2009), submarine volcanoes (Connell et al., 2009), oxygen minimum zones (Jebaraj et al., 2010), cold methane seeps (Takishita et al., 2007) and sediments (Singh et al., 2010). Previous molecular investigations of the hermatypic cold-water coral *L. pertusa* identified a fungal 18S rRNA gene sequence that was most similar to *Paecilomyces* sp. and *Acremonium* sp. (Kellogg, 2008). Histological investigations have shown fungal bioerosion in dead *L. pertusa* skeleton attributed to the filamentous fungus *Dodgella priscus* (Freiwald et al., 1997), which was visually identified but not confirmed using molecular methods. Freiwald and colleagues also described fungal hyphae associated with *Lophelia* septae, but did not identify them (Freiwald and Wilson, 1998; Wisshak et al., 2005). Herein, we present the first report of fungi cultured from living tissue of the cold-water coral *L. pertusa*. The objective of this study was to characterize non-bacterial members of the microbial community associated with *L. pertusa*. The resulting fungal isolates were phylogenetically identified and then subjected to biochemical testing to determine potential functional roles within the coral holobiont.

2. Material and Methods

2.1. Sampling sites

In conjunction with the U.S. Geological Survey DISCOVRE project (Diversity, Systematics, and Connectivity of Vulnerable Reef Ecosystems), coral samples were collected during two cruises designed to explore the ecology of *Lophelia* reefs in U.S. territorial waters. Two sites were sampled in the western Atlantic, off the Florida coast using the submersible *Johnson-Sea-Link* II in 2009. Six sites were sampled in the Gulf of Mexico using the ROV *Kraken* II from University of Connecticut in 2010. Three of those sites were in the northern Gulf of Mexico, within the Viosca Knoll Lease Block off the coast of Louisiana (VK 862, 906, 826; for further site description see: Cordes et al., 2011; Kellogg et al., 2009) and three on the West Florida Slope. A total of 27 fungal isolates were cultured from coral samples collected in both the western Atlantic (4 isolates) and Gulf of Mexico (23 isolates) and 25 were further characterized. Site locations and environmental parameters are compiled in Table 1 and Fig. 1. No fungal isolates were cultured from corals collected at the Gulf of Mexico site VK 862/906.

2.2. Fungal isolation

In order to retrieve the western Atlantic samples of *L. pertusa* without exposing them to extreme temperature gradients, the Kellogg sampler was used to maintain individual coral branches in separate, insulated compartments (see Kellogg et al., 2009 for full description). A similar method was used for the Gulf samples, with individual containers sealed after each collection to eliminate cross-contamination.

Samples were processed immediately after the dive. Using sterile techniques, a homogenate of coral tissue, mucus, and skeleton was plated onto various media. For western Atlantic collections, only Orange Serum Agar (BBL™) was used. For Gulf of Mexico collections, four types of media promoted fungal growth. OSA refers to Orange Serum Agar. FVU is Fell and van Uden agar (2% glucose, 1% peptone, 0.5% Yeast Extract, 2% agar, pH adjusted to 4.5 with lactic acid, 10 mg chloretetracycline HCl, 2 mg chloramphenicol, 2 mg streptomycin sulfate added to 1 L) (Fell et al., 1963). FBM is BBL™ Mycophil™ Agar with Low pH.

Table 1
Lophelia pertusa collection sites and corresponding environmental data.

	Dive #	Site	Fungal isolates	Latitude	Longitude	Depth (m)	Temp (°C)	Salinity (psu)	Isolates
2009 Atlantic	3705	ATL-1	2	28 46.306	79 37.0243	777	7.4	34.9	**3705k9Lp-OSAB-02**
	3712	ATL-2	2	28 19.920	79 45.4957	438	7	34.9	**3712K3Lp-OSAB-01**
2010 Gulf of Mexico	ROV-02	VK 826	2	29 10.2162	88 00.7921	490	8.6	35.0	**ROV02Q2OSA-02**
									ROV02Q2OSA-03
	ROV-03	VK 826	0	29 10.1481	88 00.8051	489	8.6	35.0	None
	ROV-04	VK 862	0	29 06.2439	88 23.0499	317	12.4	35.5	None
	ROV-05	VK 906	0	29 04.387	88 22.832	470	8.7	35.0	None
	ROV-06	VK 906	0	29 04.387	88 22.832	393	11.2	35.4	None
	ROV-07	WFS 1	5	26 12.4453	84 43.625	504	8.9	35.0	**ROV07Q1FBM-04**
									ROV07Q1OSA-02
									ROV07Q3FBM-02
									ROV07Q3FBM-03
									ROV07Q3FVU-04
	ROV-08	WFS 1	2	26 11.8795	84 43.9404	537	8.3	34.1	ROV08Q3FBM-03
									ROV08Q4FRB-03
	ROV-09	WFS 1	4	26 12.2859	84 43.9103	543	8.4	35.0	**ROV09Q1FVU-02**
									ROV09Q2FVU-01
									ROV09Q2FVU-02
									ROV09Q2FVU-03
	ROV-10	WFS 2	5	26 20.134	84 45.670	524	8.2	35.0	**ROV10Q2FRB-03**
									ROV10Q4FRB-01
									ROV10Q4FVU-05
									ROV10Q4FVU-06
									ROV10Q4FVU-07
	ROV-11	WFS 3	5	26 24.335	84 46.818	509	8.4	35.0	**ROV11Q1FRB-01**
									ROV11Q1FRB-02
									ROV11Q1FRB-04
									ROV11Q2FVU-01
									ROV11Q2FVU-02

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