Journal of Experimental Marine Biology and Ecology 484 (2016) 11–15

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Impact of explantation techniques on the microbiota of the marine sponge *Ecionemia alata*

Kyle M. Meyer [a,*], Peter Deines [a,1], Peter J. Schupp [b], Michael W. Taylor [a]

[a] School of Biological Sciences, University of Auckland, Auckland 1142, New Zealand
[b] Institute for Chemistry and Biology of the Marine Environment (ICBM), Carl-von-Ossietzky University Oldenburg, 26382 Wilhelmshaven, Germany

ARTICLE INFO

Article history:
Received 11 February 2016
Received in revised form 4 August 2016
Accepted 5 August 2016
Available online 11 August 2016

Keywords:
Marine sponge
Microbial ecology
Community ecology
Sponge growth

ABSTRACT

Marine sponges host a diverse assemblage of microorganisms, but much remains unknown about the ecology of these hosts and their microbiota. Due to variable environmental conditions in most sponge habitats, a useful way to study these animals is under controlled conditions in an aquarium setting. It is unclear, however, to what degree the process of explanting a sponge and raising it *ex situ* can perturb the structure and diversity of the microbial community within the sponge. This study assesses the impact of several sponge explantation techniques on the microbial assemblages found in the New Zealand marine sponge *Ecionemia alata*. Results indicate that there are compositional changes to the community across treatments, but that most of these changes involve shifts in the relative abundance, rather than losses or gains, of certain bacterial taxa. Alpha and beta diversity of the sponge explant microbiota is lower relative to the wild type microbiota, but across the variety of *ex situ* establishment techniques, the results are statistically indistinguishable from each other. Finally, the potential for how these changes might relate to the use of sponges for experimentation is discussed.

1. Introduction

Many marine sponges host highly diverse microbial communities that are largely distinct from those in the surrounding water column (Hentschel et al., 2006; *reviewed in* Taylor et al., 2007). This microbiota has been shown to be relatively stable through time (Hentschel et al., 2002; Schmitt et al., 2012) and contains deeply-branching microbial lineages that are only rarely found elsewhere in the surrounding environment (Taylor et al., 2013), suggesting a shared evolutionary relationship (Webster and Thomas, 2016). Sponge microbiota also produce a wide range of chemicals (*e.g.* alkaloids, peptides, polyketides, and terpenoids) with promising biotechnological applications (*e.g.* antibacterial, anticancer, antifungal, anti-inflammatory, and antiviral) (*reviewed in* Taylor et al., 2007; Selvin et al., 2010; Amarendra et al., 2013). Thus there is growing interest in the basic ecology, biogeography and specificity of sponge-microbe associations, as well as interest in the potential applications of novel bioactive compounds produced by symbionts (Taylor et al., 2007; Hentschel et al., 2012). As wild collections to obtain sufficient sponge biomass for detailed chemical and possible preclinical experiments are often limited and ecologically often not justifiable, explantation and growth of the sponge holobiont might represent one option. An important prerequisite is that microbial communities are not substantially affected by the explantation methodologies or the growth conditions.

The ability to understand sponge-microbe interactions or novel microbial physiologies associated with sponges has been hindered by difficulties in cultivating the microbial symbionts. Fortunately, many marine sponge species are amenable to transplantation by exhibiting fast healing and regrowth of their outer dermal layer (pinacoderm) after removal from the reef. This is due in part to their relatively simple anatomy which is constructed of in some cases as few as 10 different cell types, some of which are totipotent (Bergquist, 1978; Simpson, 1984). Many sponge species are also rather abundant and widespread globally (Bergquist, 1978), making them relatively accessible in most coastal regions worldwide. These characteristics make sponges an attractive model to study invertebrate host-microbe interactions and address emerging frontiers (recently *reviewed in* Webster and Thomas, 2016), as well as to screen for novel microbe-derived chemical compounds.

The marine sponge *Ecionemia* (formerly *Ancorina*) *alata* is commonly found in the temperate and sub-tropical coastal waters of New Zealand. It is generally thought that dominant sponge species such as *E. alata* exert a strong influence on the planktonic community of the surrounding water column and are therefore of ecological importance for waters surrounding their habitat. This sponge is considered a high microbial abundance (HMA) sponge and is host to a diverse assemblage of microbes (Kamke et al., 2010). This sponge has also been the focus of a number of microbial ecology studies that have focused on the activity (Kamke et al., 2010), membership, and specificity of its microbiota

* Corresponding author at: Institute of Ecology and Evolution, Department of Biology, University of Oregon, Eugene, OR 97403, USA.
E-mail address: kmeyer@uoregon.edu (K.M. Meyer).
[1] Present address: Zoological Institute, Christian-Albrechts-University Kiel, Kiel 24118, Germany.

http://dx.doi.org/10.1016/j.jembe.2016.08.003
0022-0981/

(Schmitt et al., 2012; Taylor et al., 2013; Cárdenas et al., 2014). Despite being the focus of numerous studies, there have been limited efforts to date to establish *E. alata* in aquarium conditions for manipulative experiments. The ability to maintain sponge explants *ex situ* provides a powerful system to explore, for example, the impacts of potential pathogens or changing environmental conditions (*e.g.* temperature, nutrient load on the sponge holobiont). It also provides an opportunity to enhance production of bioactive compounds in the holobiont by optimizing aquarium conditions or by inducing compound production (Rohde et al., 2015).

It is important to understand how the microbiota of a sponge is impacted by explantation and establishment in aquarium conditions. Several aspects of the transfer from the wild could alter the microbiota of marine sponges including cutting the tissue, the location where the explants are allowed to heal, the aquarium water source, and the duration of time *ex situ*. To date, relatively few studies have assessed the impact of explantation and aquarium establishment methods on the microbiota. Among the few existing studies, there are mixed results, with some demonstrating changes to the community (Mohamed et al., 2008a, 2008b; Isaacs et al., 2009) and others reporting little or no change (Gerçe et al., 2009; Webster et al., 2011). This could be related to the explantation technique being used, the sponge species being tested, or the microbiological survey method being applied. Most of these studies used clone libraries, molecular fingerprinting techniques, or cultivation-dependant techniques which provide a fairly coarse and/or potentially biased perspective on the community. Thus, there is a paucity of studies assessing the impacts of explantation and aquarium establishment on the sponge microbiota and even fewer studies using next-generation sequencing (which provides a deeper survey of the microbial community).

This study tests the impact of explantation and aquarium establishment on the microbiota of the New Zealand sponge *Ecionemia alata*. The goal of the study was to develop a methodology for growing this sponge *ex situ* for future manipulative experiments, while minimally impacting the native microbiota. A variety of sponge explantation strategies (*e.g.* recovery and growth in *in situ* reef cages, *ex situ* aquarium establishment, and combinations thereof) were tested, as well as a variety of growth times. Results indicate that most of the sponge explantation methods had little to no impact on bacterial taxonomic and phylogenetic diversity and that there was very little change to the community structure. Finally, the application of certain aspects of these methodologies to sponge explantation systems is discussed.

2. Materials and methods

2.1. Collection

Specimens of *Ecionemia alata* were collected from 3 m depth by SCUBA from Jones Bay, Tawharanui Peninsula, New Zealand (36° 22.143′ S, 174° 50.247′ E) in June 2010. The sponges were cut from the rocky substratum using a dive knife, then cut into roughly 8 cm^3 pieces. Explants were transferred from the ocean to the nearby University of Auckland Leigh Marine Laboratory in natural seawater inside sterile Ziplock bags with minimal exposure to direct sunlight, or left in the field. Mean solar radiation at 5 m depth at Jones Bay in June (austral winter) is approximately 0.6 MJ/m^2/day, with mean seawater temperatures of 15–16 °C.

2.2. Experimental design

Explants of *E. alata* were raised using several methods: growth in open ocean cages, growth in flow-through aquaria, and various combinations thereof (Table 1). Those raised using open ocean cages were distributed throughout three racks made of plastic mesh, zip-tied to bricks placed on rocky substratum at a depth of 3 m. The three locations were all within 15 m of each other. A subset of these sponge explants was analyzed directly after 10 and 30 days in the ocean. The remainder of the sponge explants undergoing open ocean explantation were either kept 10 days in the ocean then transferred to aquaria for 10 or 30 days, or kept 30 days in the ocean then 10 days in aquaria. Sponge explants that were taken directly to aquaria from the wild were sampled after 10 and 30 days. The 10–40 day time interval was chosen based on past observations that *E. alata* clones were able to heal and begin to regrow during this amount of time and could therefore be used for experimentation soon thereafter.

Table 1
Time intervals for sponge clone explantation. Sponge clones raised in both the ocean (O) and aquarium (A) systems always started in the ocean, then were transferred to aquaria (WT – wildtype donor sponge).

Treatment	Time (days) grown in ocean cages	Time (days) grown in aquarium	Total time (days) since explantation
WT	0	0	0
A10	0	10	10
O10	10	0	10
O10A10	10	10	20
A30	0	30	30
O30	30	0	30
O10A30	10	30	40
O30A10	30	10	40

Three outdoor (1300 L) flow-through polypropylene aquaria were used at the Leigh Marine Laboratory. Incoming seawater was filtered to 200 μm. To minimize algal growth and fouling in the aquaria, counter-balanced dump tanks (Barr et al., 2008) were used to administer the filtered seawater periodically. Incoming seawater was dumped at a rate of roughly 35 L/min. Sponge explants were kept in hydroponic cups ziptied to a plastic mesh anchored to the bottom of the tank (Supplemental Fig. 1). Mean solar radiation reaching the sponge explants in the tanks was approximately 2.1 MJ/m^2/day (shade cloths intercepting 70% of light were placed over each tank), and mean seawater temperature was 15.9 °C.

2.3. DNA extraction, PCR, and sequencing

Explant samples, including portions of both the mesohyl (inner tissue) and pinacoderm (outer tissue), were frozen at −20 °C until the end of the experiment. They were then freeze-dried in a Virtis freeze-drier at −65 °C for 24 h. Freeze-dried sponge tissue was crushed with a sterile pestle and mortar. DNA was extracted following a bead-beating protocol whereby 5 mg of crushed, freeze-dried sponge tissue was combined with 1 mL of extraction buffer (400 μL 6.25 M ammonium acetate; 100 μL Tris (pH .0); 40 μL 0.5 M EDTA; 460 μL molecular grade water), 200 μL of 0.1 mm zirconia/silica beads (BioSpec Prod. Inc.), 15.0 mg polyvinylpolypyrrolidone (PVPP), and 300 μL of chloroform:isoamyl alcohol (24:1) (Taylor et al., 2004). Bead beating was performed using a BIO 101 Savant FastPrep machine, followed by centrifugation (30 min, 15,000*g*, at room temperature) and collection of the supernatant. This was then precipitated overnight (−20 °C) with 3 M sodium acetate and isopropanol, followed by centrifugation for 30 min (15,000*g*, 4 °C). Pellets were washed twice with 70% ethanol, dried, and re-suspended in 30 μL Ultrapure water (GIBCO). Approximately 1–3 ng of extracted DNA was used as a template for the polymerase chain reaction (PCR) on an Eppendorf Mastercycler Gradient machine using previously described primers (533F-907R) and thermal cycling conditions (Simister et al., 2012). The sizes of the PCR products were then verified by electrophoresis on a 1% agarose gel at 90 V for 40 min. Approximately 2000–3000 ng of each PCR product was purified using AMPure magnetic beads (Agencourt), the sequenced by Macrogen (Seoul, South Korea) using the Roche GS FLX Titanium pyrosequencing platform.

2.4. Bioinformatics and statistical analysis

The pyrosequencing sff files were converted to FASTA files and demultiplexed in QIIME (Caporaso et al., 2010) before quality filtering.