Saline-Saturated DMSO-EDTA as a Storage Medium for Microbial DNA Analysis from Coral Mucus Swab Samples

Lisa A. May, Julie L. Higgins, and
Cheryl M. Woodley

February
2011

NOAA Technical Memorandum NOS NCCOS 127
NOAA Technical Memorandum CRCP 15

This report has been reviewed by the National Ocean Service of the National Oceanic and Atmospheric Administration (NOAA) and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for their use by the United States government.

Citation for this Report

May, L. A., Higgins, J. L., and Woodley, C. M. 2011. Saline-Saturated DMSO-EDTA as a Storage Medium for Microbial DNA Analysis from Coral Mucus Swab Samples. NOAA Technical Memorandum NOS NCCOS 127 and CRCP 15. 14 pp.

Saline-Saturated DMSO-EDTA as a Storage Medium for Microbial DNA Analysis from Coral Mucus Swab Samples

Lisa A. May[1], Julie L. Higgins[1], and Cheryl M. Woodley[2]

[1]JHT, Inc., NOAA, National Ocean Service
National Centers for Coastal Ocean Science
Center for Coastal Environmental Health and Biomolecular Research
Hollings Marine Laboratory
331 Fort Johnson Road
Charleston, South Carolina 29412

[2]NOAA, National Ocean Service
National Centers for Coastal Ocean Science
Center for Coastal Environmental Health and Biomolecular Research
Hollings Marine Laboratory
331 Fort Johnson Road
Charleston, South Carolina 29412

NOAA Technical Memorandum NOS NCCOS 127
NOAA Technical Memorandum Coral Reef Conservation Program 15

February 2011

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Introduction:

The mucus surface layer of corals plays a number of integral roles in their overall health and fitness. This mucopolysaccharide coating serves as vehicle to capture food, a protective barrier against physical invasions and trauma, and serves as a medium to host a community of microorganisms distinct from the surrounding seawater. In healthy corals the associated microbial communities are known to provide antibiotics that contribute to the coral's innate immunity and function metabolic activities such as biogeochemical cycling.

Culture-dependent (Ducklow and Mitchell, 1979; Ritchie, 2006) and culture-independent methods (Rohwer, et al., 2001; Rohwer et al., 2002; Sekar et al., 2006; Hansson et al., 2009; Kellogg et al., 2009) have shown that coral mucus-associated microbial communities can change with changes in the environment and health condition of the coral. These changes may suggest that changes in the microbial associates not only reflect health status but also may assist corals in acclimating to changing environmental conditions. With the increasing availability of molecular biology tools, culture-independent methods are being used more frequently for evaluating the health of the animal host. Although culture-independent methods are able to provide more in-depth insights into the constituents of the coral surface mucus layer's microbial community, their reliability and reproducibility rely on the initial sample collection maintaining sample integrity. In general, a sample of mucus is collected from a coral colony, either by sterile syringe or swab method (Woodley, et al., 2008), and immediately placed in a cryovial. In the case of a syringe sample, the mucus is decanted into the cryovial and the sealed tube is immediately flash-frozen in a liquid nitrogen vapor shipper (a.k.a., dry shipper). Swabs with mucus are placed in a cryovial, and the end of the swab is broken off before sealing and placing the vial in the dry shipper. The samples are then sent to a laboratory for analysis. After the initial collection and preservation of the sample, the duration of the sample voyage to a recipient laboratory is often another critical part of the sampling process, as unanticipated delays may exceed the length of time a dry shipper can remain cold, or mishandling of the shipper can cause it to exhaust prematurely. In remote areas, service by international shipping companies may be non-existent, which requires the use of an alternative preservation medium. Other methods for preserving environmental samples for microbial DNA analysis include drying on various matrices (DNA cards, swabs), or placing samples in liquid preservatives (e.g., chloroform/phenol/isoamyl alcohol, TRIzol reagent, ethanol). These methodologies eliminate the need for cold storage, however, they add expense and permitting requirements for hazardous liquid components, and the retrieval of intact microbial DNA often can be inconsistent (Dawson, et al., 1998; Rissanen et al., 2010).

A method to preserve coral mucus samples without cold storage or use of hazardous solvents, while maintaining microbial DNA integrity, would be an invaluable tool for coral biologists, especially those in remote areas. Saline-saturated dimethylsulfoxide-ethylenediaminetetraacetic acid (20% DMSO-0.25M EDTA, pH 8.0), or SSDE, is a solution that has been reported to be a means of storing tissue of marine invertebrates at ambient temperatures without significant loss of nucleic acid integrity (Dawson et al., 1998, Concepcion et al., 2007). While this methodology would be a facile and inexpensive way to transport coral tissue samples, it is unclear whether the coral microbiota DNA would be adversely affected by this storage medium either by degradation

of the DNA, or a bias in the DNA recovered during the extraction process created by variations in extraction efficiencies among the various community members. Tests to determine the efficacy of SSDE as an ambient temperature storage medium for coral mucus samples are presented here.

Experiment 1. Test of DNA Recovery and PCR integrity of *Acropora palmata* Mucus Swab Samples

Coral mucus was collected from five *Acropora palmata* colonies exhibiting both healthy and disease states using DNA collection swabs (Catch-All Sample Collection Swabs, Epicentre Biotechnologies, Madison, WI) at Hawksnest Bay, St. John, US Virgin Islands on 9 June 2008 (a total of 10 collected samples). Two swabs were collected per coral, one from the disease lesion and one from the healthy-appearing tissue distant from the lesion. Individual swabs were placed in 2.0 ml cryovials containing 1.0 ml SSDE solution (sufficient to cover the applicator end of the swab). The swab handle was snapped off prior to capping the vial and all samples were shipped to the Hollings Marine Laboratory in Charleston, SC for analysis (Table 1).

Table 1. Acropora palmata coral colony mucus swab sample detail, Hawksnest Bay, St. John, US Virgin Islands. "Healthy" refers to normal-appearing tissue on a part of the colony away from the disease lesion.

Vial #	Colony #	Healthy(H)/Diseased (D)
1	1	H
2	1	D
3	2	H
4	2	D
5	3	H
6	3	D
7	4	H
8	4	D
9	5	H
10	5	D

DNA isolations were performed on samples after 3 weeks at room temperature. To determine the best DNA isolation protocol for optimal nucleic acid recovery, three methods were employed: 1) a bacterial pellet recovery and extraction, 2) swab extraction, and 3) swab extraction with supplemental poly A and final elution in 10 mM Tris-1mM EDTA (TE, pH 8.0). Sample vials 1-4 (healthy and diseased samples from colonies 1 and 2) were vortexed for 3 min to release bacterial cells from the swab into the suspension and then centrifuged at 14,000 rcf for 15 min at 4ºC. The swab was carefully removed; the end of the swab then was cut off with sterile scissors and placed into a separate sterile 1.5 ml Eppendorf tube for the direct DNA isolation from any adherent cells. From the initial centrifugation, most of the supernatant was aspirated from the cryovial, leaving approximately 200 µl over the bacterial pellet. This

remaining volume was used to resuspend the bacterial pellet (not visible) for DNA extraction. Healthy and diseased samples from colonies 3-5 (healthy and diseased, vials 5-10) were subjected to a swab extraction with supplemental poly A RNA in the extraction mixture and elution with TE. From this point all samples were processed in a similar manner. The DNA was extracted from each sample set (pellet or swab) using the Fast DNA Kit for Soil (MP Biomedicals, LLC, Solon, OH), which incorporates mechanical disruption of the bacterial cells (bead beating). Tubes were designated 1p, 2p, 3p, 4p for the pellet extractions, and 1s, 2s, 3s, 4s for the swab extractions. A fluorescence-based DNA quantification technique (Quant-iT DNA Assay kit, High Sensitivity, Invitrogen/Molecular Probes, Eugene, OR) using a Qubit fluorometer revealed that almost no DNA was recovered from samples targeting only the bacterial pellets; however a sufficient amount of DNA was recovered when extractions were conducted using the intact swab, indicating that the mucus associated bacteria were tightly bound to the DNA swab (Table 2). Modifications of the DNA purification protocol incorporated supplemental poly A RNA (200 mg) in each sample to facilitate DNA recovery (Shaw, et al., 2009), and substitution of 10 mM Tris-1mM EDTA (TE, pH 8.0) for the kit-supplied "DES solution" to inhibit potential DNA degradation (Rozman and Komel 1994). All samples were used in a PCR assay (polymerase chain reaction) for denaturing gradient gel electrophoresis (DGGE) analysis. It should be noted that varying the amount of poly A added to the extraction tubes can significantly alter DNA recovery amounts, producing spurious results (Figure 1). With a typical recovery of microbial DNA from mucus in the range of 1 µg, the contribution of the poly A may be as much as 4% of the total quantity measured. However if the amount of supplemental poly A RNA is decreased to 20 µg, the contribution of the RNA to the total DNA increases to 8%, which may have negative effects on downstream applications (PCR).

Table 2. DNA recovery of SSDE samples from coral mucus swabs stored for 3 weeks at room temperature. Samples 1p-4p were from vortexing and centrifuging the swab to dislodge and pellet sample bacteria prior to DNA purification. Samples 1s-4s were swab DNA extractions without prior bacterial centrifugation, but with added poly A RNA to facilitate DNA precipitation.

Sample	Disease state (H/D)	DNA Concentration (ng/mL)
1p	H	< 10
1s	H	20
2p	D	< 10
2s	D	< 10
3p	H	< 10
3s	H	35
4p	D	< 10
4s	D	12
5	H	264
6	D	100
7	H	404
8	D	330
9	H	5600
10	D	316

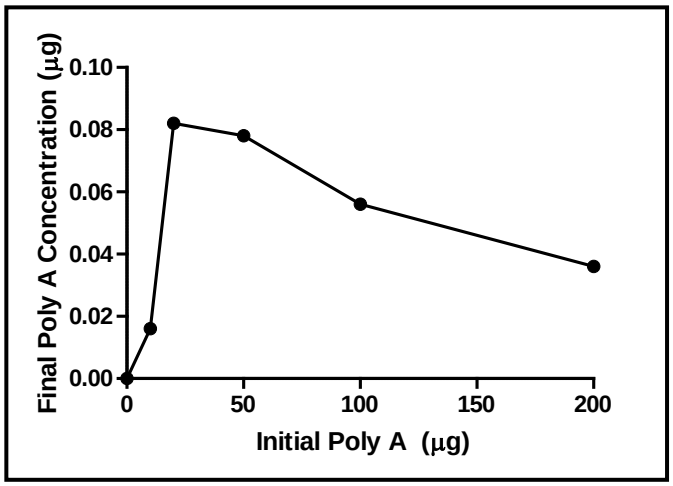

Figure 1. Poly A RNA detection following FastDNA® Spin Kit for Soil extraction. To determine the potential contribution of poly A RNA to DNA quantification, varying amounts (0, 10, 20, 50, 100 and 200 µg) of poly A RNA were added to 200 µl sterile water. These samples were purified using the MP Biomedicals kit (standard coral mucus extraction protocol). Following extraction, the amount of poly A detected using the Qubit fluorometer was determined. At 200 µg initial concentration (our standard addition of poly A), only 0.036 µg was detected.

The PCR amplification mixture contained 1 µl sample DNA, 5 µl 10X ExTaq buffer, 20 µM each primer, 1 µl dNTP mixture, 0.25 µl ExTaq polymerase, and sterile water to a final volume of 50 µl. As a positive control, a defined mixture of bacterial DNA from four putative coral pathogens (*Serratia marcescens, Vibrio shiloi, Vibrio coralliilyticus,* and *Aurantimonas coralicida*) was used. The negative control incorporated sterile water in the PCR mixture in place of template DNA. Bacterial specific primers for the 16S rRNA gene were 911F 5'-TCA AAT GAA TTG ACG GGG GC-3' (Maidak, 1996) and 1406R 5'-CGC CCG CCG CGC CCC GCG CCC GGC CCG CCG CCC CCG CCC CAC GGG CGG TGT GTA C-3' (Lane, 1988) with GC clamp (underlined). Touchdown PCR was performed as follows: 95 ºC for 5 min; 20 cycles of 94 ºC for 1 min, 65 ºC (-0.5 ºC /cycle) for 1 min, 72 ºC for 1 min; 20 cycles of 94 ºC for 1 min, 55 ºC for 1 min, 72 ºC for 1 min; terminal extension at 72 ºC for 10 min. DGGE gel (30-60% denaturant, 7% polyacrylamide) was run for 16.5 hours at 60V (60ºC) using the DCode Universal Mutation Detection System (Bio-Rad Laboratories; Hercules, CA USA). The gel was stained with ethidium bromide (1 µg/mL) and destained with TAE buffer (40 mM Tris base, 20 mM glacial acetic acid, 1 mM EDTA) prior to visualization. A digital image was captured using the FluorChem™ 8900 fluorescent imager (Alpha Innotech, San Leandro, CA), equipped with a UV transilluminator (365nm).

The resulting denaturing gradient gel (Figure 2) indicates that DNA recovery was sufficient only for healthy samples (1 and 3) using the pellet or swab purification methodologies, while no results could be obtained from the diseased samples in this group (even numbers). However it must be noted that more consistent results were obtained when the swabs were extracted with the addition of 200µg poly A RNA (samples 5-10). This method also yielded more bands per

sample, indicating increased diversity of mucus microbes revealed using this protocol. The lane 9 sample (healthy swab from colony 5) had the highest DNA recovery, yet poor amplification yield as shown on the denaturing gradient gel. This illustrates that there is an optimum DNA template concentration range for the PCR, and that too much template may produce effects similar to insufficient template. The experimental results show that supplemental poly A in the DNA extraction mixture greatly improves microbial DNA recovery from the samples, and that microbial DNA can be recovered after sample storage in SSDE at room temperature after about 3 weeks. However it is not clear how this compares to storing the samples in liquid nitrogen.

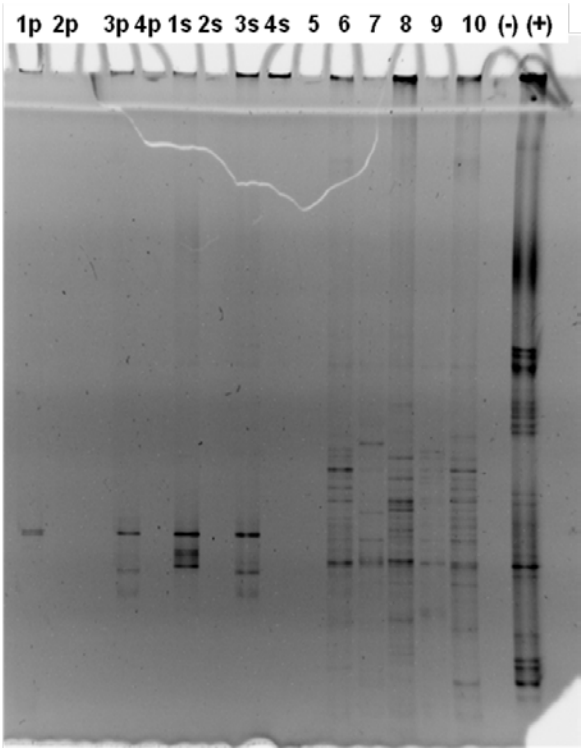

Figure 2. DGGE results of the DNA extraction test for SSDE-stored coral mucus swabs. Lanes 1p-4p: DNA from bacterial pellet from coral colonies 1 and 2 (healthy and diseased), Lanes 1s-4s: DNA from swab extractions from coral colonies 1 and 2 (healthy and diseased), Lanes 5-10: DNA from coral colonies 3-5 (healthy and diseased) swabs using added poly A RNA, Lane (-): PCR negative control, Lane (+): PCR positive control using defined mixture of bacterial DNA.

Experiment 2. Long-term SSDE Coral Mucus Swab Storage

Six coral mucus samples collected with DNA swabs were taken from a single colony of *Montastraea annularis* located in the NOAA Coral Culture and Collaborative Research Facility, Charleston, SC. Samples were placed immediately into one of 6 screw-capped cryovials. Three cryovials contained 1.0 ml SSDE (20% DMSO-0.25M EDTA, pH 8.0, saturated with NaCl)

storage solution and were stored at room temperature (22ºC) (D=SSDE samples). The three remaining tubes without SSDE solution were frozen in liquid N_2, then transferred to a -80˚C freezer for long term storage (L=liqN_2 samples). After 4.5 months, the six coral swab samples were subjected to a DNA isolation protocol using the methodology determined to maximize yield in the previous study (including the addition of poly A RNA to the extraction mixture). DNA recovery was determined using the Quant-iT DNA Assay kit, High Sensitivity (Invitrogen/Molecular Probes, Eugene, OR) on a Qubit fluorometer. Sufficient DNA recovery occurred with all samples (Table 3).

Table 3. DNA recovery from SSDE samples stored 4.5 months at room temperature. Samples L1-L3 were stored in liquid nitrogen for 4.5 months and samples D1-D3 were stored in saline saturated DMSO-EDTA (SSDE) solution for 4.5 months at room temperature (22ºC). DNA recovery was sufficient for use in a PCR for all samples.

Sample	DNA concentration (ng/mL)
L1	9000
L2	12000
L3	9300
D1	7500
D2	7000
D3	700

Samples were PCR-amplified for DGGE using approximately 10 ng DNA according to the protocol detailed in Experiment 1. Following the PCR, 100 ng of amplified product was loaded onto a denaturing gradient gel (7% polyacrylamide, and 35-60% denaturant) and electrophoresed at 60˚C and 60V for 16.5h. The denaturing gradient was altered slightly (30-60% to 35-60%) to improve band resolution for the samples. The gel was stained for 20 min in 1.0 µg/mL ethidium bromide in 1 X TAE buffer for 20 min and destained in buffer alone for 20 min prior to imaging on a UV transilluminator using the FluorChem™ 8900. Amplified banding patterns were more prominent in the samples stored in liquid nitrogen (Figure 3). It is possible that the SSDE solution may not be entirely eliminated in the DNA extraction process and may inhibit PCR to a small extent, or prevent accurate DNA quantification. Adjusting the template concentration for the SSDE sample PCR could eliminate these problems.

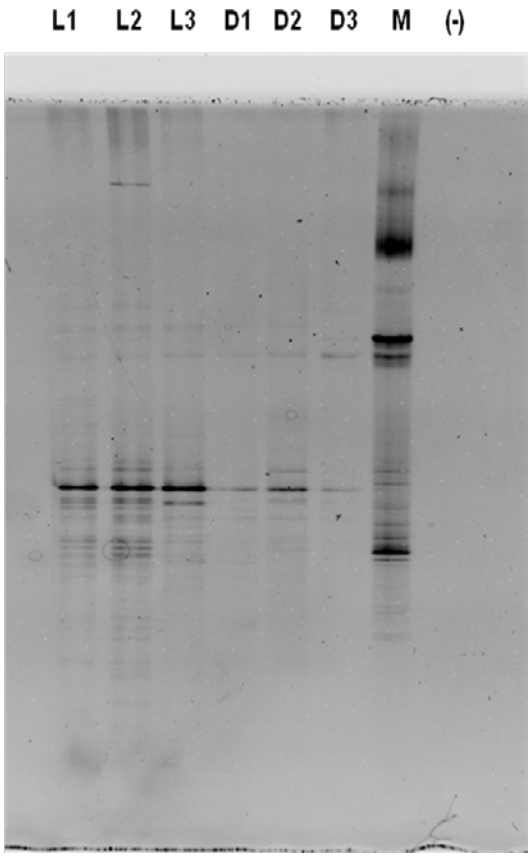

Figure 3. DGGE results of long-term storage experiment. Samples stored in liquid nitrogen (L1-L3) had stronger banding patterns than samples stored in SSDE (D1-D3). Lane M contains a defined mixture of known bacteria from *Serratia marcescens, Vibrio shiloi, Vibrio coralliilyticus,* and *Aurantimonas coralicida* as a positive control. The negative control (sterile water) is designated (-).

Experiment 3. Temporal Study of SSDE as a Storage Medium for Coral Mucus Swab Samples.

Nine swab samples from different colony areas (approximately 2 cm^2) were taken from the cultured *Montastraea annularis* used in Experiment 2 (Figure 4). Swab samples for standard long-term storage (n=4) were immediately placed in liquid nitrogen (liqN2), and transferred to a -80°C freezer. SSDE swab samples (n=4) were immediately placed in 1.0 ml solution, and stored at room temperature (22ºC). The final sample taken was placed on ice, and used for a DNA purification control within 20 min.

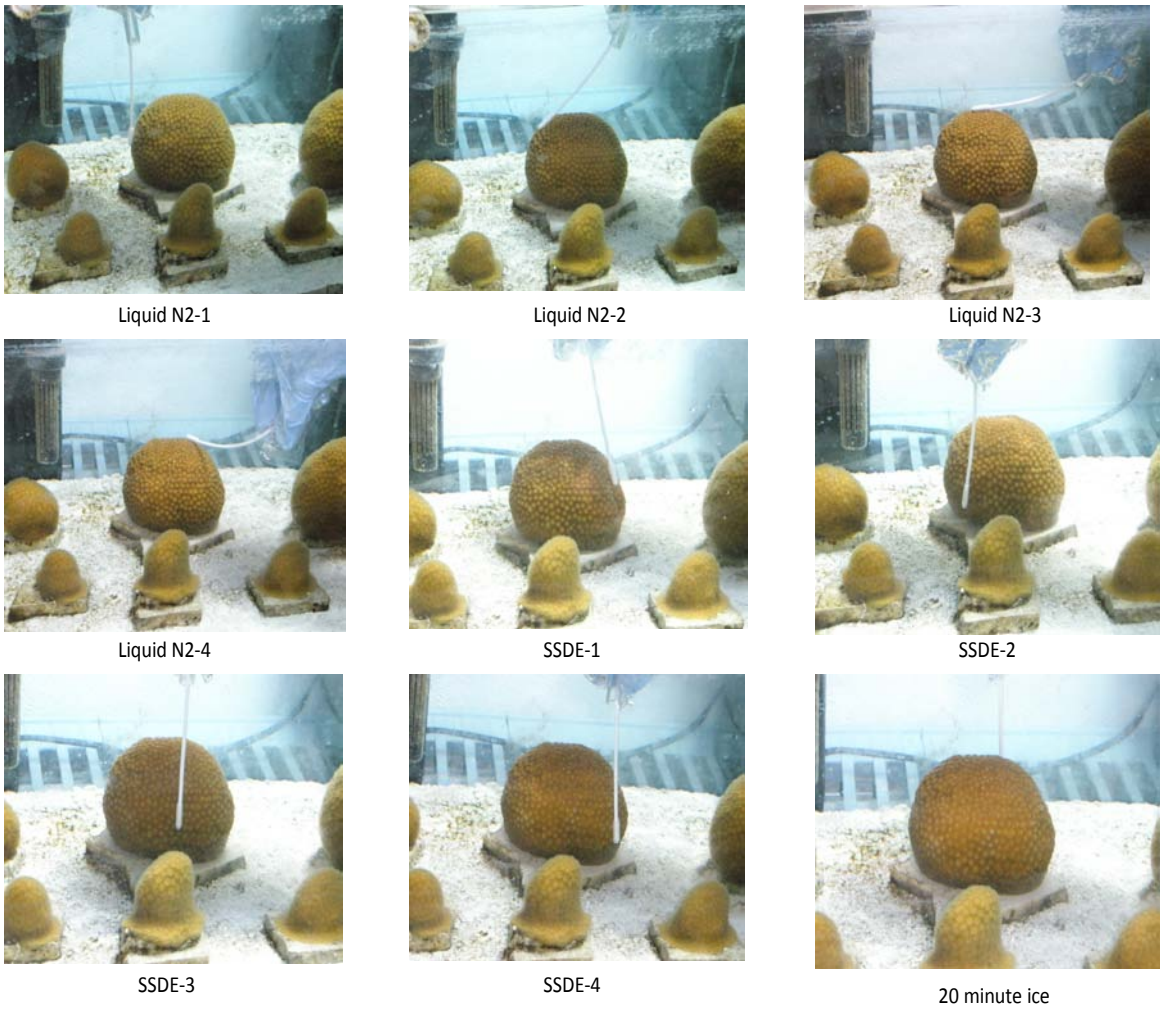

Figure 4. Swab method of *Montastraea annularis* from the NOAA Coral Culture and Collaborative Research Facility. Mucus swabs were taken at nine different sites on the colony, and used in a comparative time course study of liquid nitrogen and saline saturated DMSO-EDTA solution storage methods. *Photos courtesy of Thomas Bartlett.*

At 1 week, 2 weeks, 4 weeks, and 8 weeks post-sampling, DNA purifications were prepared from one liqN2 and one SSDE sample using the FastDNA® SPIN for Soils (MP Biomedicals, LLC, Solon, OH) according to the protocol developed above for maximum recovery (i.e., extraction from the swab alone and addition of poly A RNA). DNA concentration was quantified with the Quant-iT DNA Assay kit, High Sensitivity (Invitrogen/Molecular Probes, Eugene, OR) on a Qubit fluorometer (Table 4). The sample used immediately in a DNA extraction resulted in the highest amount of nucleic acid recovered from any sample. Low recovery of DNA occurred from the 1-week SSDE sample, most likely as an artifact resulting from a DNA pellet lost during the drying process. Nucleic acid samples were stored at -20°C until all had been processed.

Table 4. DNA recovery from coral swab SSDE samples in the temporal study. *Low recovery of DNA from this sample is likely an artifact resulting from a DNA pellet lost during the drying process.

Sample	DNA Recovery (ng/mL)
Immediate prep	8800
1 wk liquid N_2	1400
1 wk SSDE	70*
2 wk liquid N_2	1300
2 wk SSDE	2900
4 wk liquid N_2	2700
4 wk SSDE	1500
8 wk liquid N_2	3100
8 wk SSDE	1300

Sample DNA was amplified using primers specific for the 16S rRNA gene with the following reaction parameters: 5 µl 10 X buffer, 5 µl 25 mM $MgCl_2$, 4 µl dNTP solution, 1 µl 911F (20 pmol), 1 µl 1406R-GC (20 pmol), and 0.25 µl ExTaq polymerase. The remaining reaction volume contained 1, 2, or 5 µl template DNA (2, 5, or 10 µl for 1-week SSDE sample) and sterile water to 50 µl. The amplification parameters were: 94°C for 5 min; 20 cycles of: 94°C for 1 min, 65°C for 1 min (-0.5°C/cycle), 72°C for 1 min; and 20 cycles of: 94°C for 1 min, 55°C for 1 min, 72°C for 1 min; and a final extension of 72°C for 10 min. Five microliters of each reaction (one-tenth volume) was checked on a 1%-TAE agarose gel (75 ml volume) with 0.5 µg/mL ethidium bromide at 100V for 35 min. DNA size markers (Promega 100 bp DNA ladder) were used for size and concentration estimation. Gel was imaged on a UV transilluminator using the FluorChem™ 8900 Digital Imaging System. Agarose gel electrophoresis of the 16S rDNA PCR products indicated that all coral mucus samples amplified (Figure 5). Variability in product yield among triplicate samples is due to the different amounts of template used in the reaction mixture.

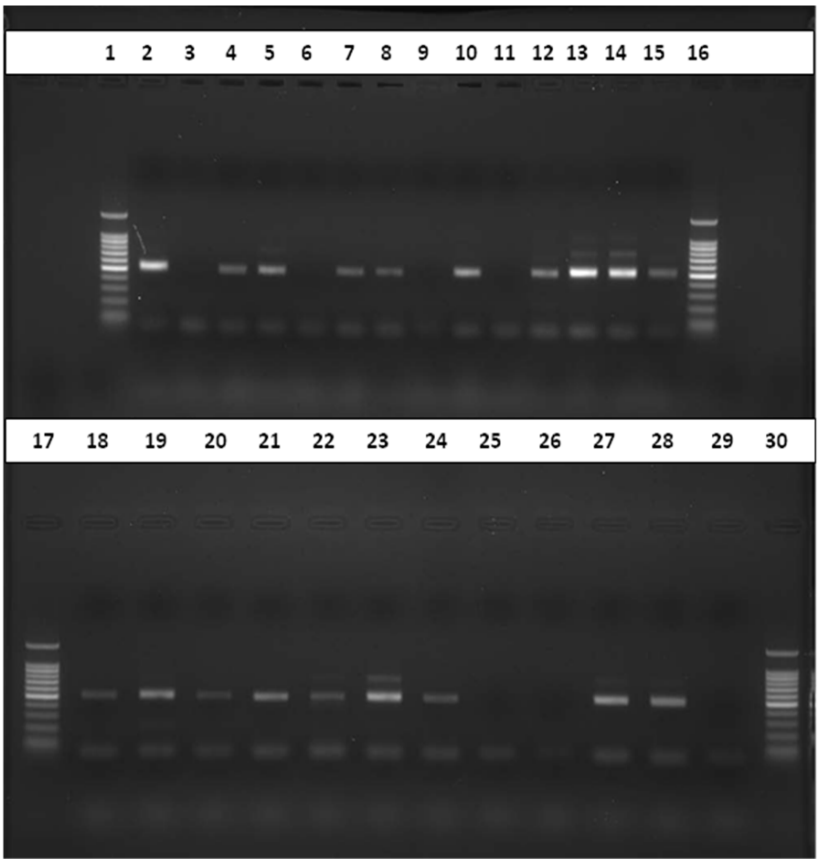

Figure 5. Amplification results of DGGE PCR. Lanes 1, 16, 17 and 30: Promega 100 bp DNA ladder, Lanes 2: Immediate DNA prep, Lane 3: negative PCR control, Lanes 4-6: 1-week LN_2, Lanes 7-9: 1-week SSDE, Lanes 10-12: 2-week LN_2, Lanes 13-15: 2-week SSDE, Lanes 18-20: 4-week LN_2, Lanes 21-23: 4-week SSDE, Lanes 24-26: 8-week LN_2, Lanes 27-29: 8-week SSDE. Triplicate samples have increasing DNA template in the PCR. 16S DNA from all samples was successfully amplified.

Between 20-30 microliters of amplified product from each sample were run on a 7% polyacrylamide, 35-60% denaturing gel at 60V and 60°C for 16.5 h. The gel was stained in ethidium bromide (50 µg/mL in 1 X TAE) for 30 min and destained in 1 X TAE 5 min prior to image capture on the FluorChem™ 8900 Digital Imaging System. The one-week coral mucus samples (LN_2 and SSDE) had dissimilar banding patterns on the DGGE gel (Figure 6). This is attributed to the partial pellet loss for the one-week SSDE sample in the DNA purification step. The SSDE-stored coral mucus samples were highly similar to LN_2-stored samples for all other time points, both in terms of DNA recovery (Table 4) and banding patterns (Figure 6). While none of the archived samples exhibited the microbial diversity shown in the sample immediately processed, SSDE has been demonstrated to be a suitable medium for storage of coral mucus swab samples for use in microbial community analyses.

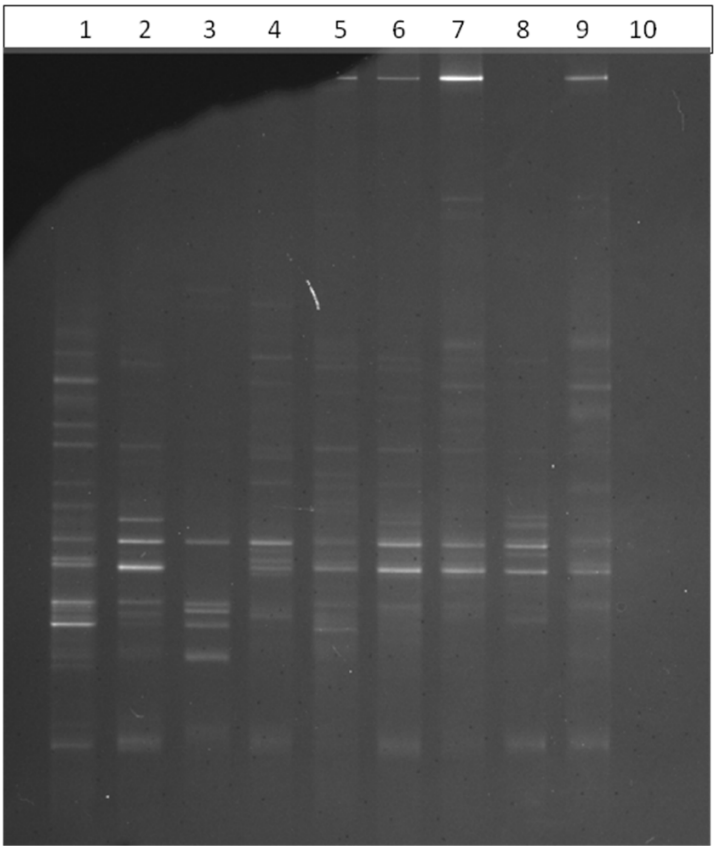

Figure 6. Results of temporal storage study using denaturing gradient gel electrophoresis. Lane 1: Immediate DNA prep (20 min ice), Lane 2: 1-week LN_2, Lane 3: 1-week SSDE, Lane 4: 2-week LN_2, Lane 5: 2-week SSDE, Lane 6: 4-week LN_2, Lane 7: 4-week SSDE, Lane 8: 8-week LN_2, Lane 9: 8-week SSDE, Lane 10: negative PCR control.

Discussion

DNA-based analysis of coral mucus microbial communities has become increasingly prevalent in recent years. This approach can give researchers a broad insight into the microbial ecosystem and the influences the community may have on coral health. However, isolation of microbial DNA from the coral surface mucopolysaccharide layer (SML) of sufficient quality for downstream applications (PCR) has been problematic. The SML is a nutrient-rich matrix composed of proteins, lipids and carbohydrates (Ducklow and Mitchell 1979). Various methods have been presented to minimize unwanted effects from coprecipitation of the DNA with polysaccharide byproducts, while improving DNA yield (Rohwer, et al., 2001; Harder et al., 2002; Kellogg, 2004). Mechanical disruption ensures that microbes with cell walls (Gram positive bacteria and fungi) are represented in a community profile. Additionally, incorporation of supplemental poly A RNA to the extraction mixture can facilitate precipitation of the

microbial DNA, increasing yield from a sample anticipated to have low numbers of microorganisms (Shaw, et al., 2009). Finally, selective alcohol precipitation and the use of a metal-chelating agent such as EDTA in the elution buffer, minimizes the presence of contaminating polysaccharides and the activity of degrading enzymes in solution. Once purified and in sufficient quantity, microbial DNA can be used for a community analysis.

The results of our study indicate that SSDE is a viable preservative for coral mucus samples taken by the DNA swab method after 4 months of room temperature storage. This preservation method also allows isolation of sufficiently pure DNA to support downstream PCR-based applications. It is important to note that most bacteria adhere to the sampling swab and are not easily removed, so any microbial DNA extraction protocol from DNA collection swabs, should incorporate the swab. Our results also indicate that high-yield PCR products from SSDE-stored coral mucus may require different amplification parameters from those of LN_2-stored samples. Coral mucus extractions performed within 20 minutes of sampling produced the highest amount of microbial DNA yield from any sample. Supplemental poly A RNA (200µg/sample tube) greatly improved yield of PCR-quality microbial DNA from coral mucus.

Considerations and Recommendations:

- Microbial DNA was successfully recovered from coral swab samples (*A. palmata* and *M. annularis*) up to 4.5 months after storage in SSDE. DNA preparations included bead beating the swab with 200 µl of SSDE storage solution using the MP Biologicals, LLC FastDNA® Kit for Soil. Additional poly A RNA (200 mg) improved microbial DNA recovery for PCR, especially for samples from diseased tissue.
- Amplification of microbial DNA from SSDE-stored samples was successful for all storage times tested. The PCR results indicate that purified DNA from SSDE-stored samples may be of lower quality than DNA from LN_2-stored samples. Variability of the microbial community at earlier time points of the SSDE-stored samples may be due to 1) differences in the coral microbial community sampled, 2) a result of some DNA degradation following purification (as the 1- and 2-week DNA samples were stored the longest prior to PCR analysis), 3) inherent PCR amplification bias, 4) differences in DNA recovery, or 5) dilution of bacteria in the SSDE solution. Liquid nitrogen stored samples were similarly variable over time.
- It is recommended that once coral mucus samples are extracted, DNA solutions not be stored at 4ºC for longer than 24 hours prior to amplification, as it is possible that the DNA extraction process may not eliminate all compounds which could degrade the nucleic acid. If it is anticipated that the purified DNA will not be analyzed by PCR within this time period, the DNA should be precipitated with a high salt-ethanol (molecular biology-grade) solution, centrifuged to pellet the nucleic acid, and the pellet washed with 70% ethanol. The DNA can be archived (-20ºC) as a lyophilized pellet (for detailed protocol, see Sambrook and Russell, 2001). Alternatively, amplification for temporal studies can be executed immediately and the PCR products stored at -20ºC until all samples are ready for electrophoresis.

- Modifications to the PCR mixture which could improve yield for troublesome DNA template samples include: increasing primer concentration, the addition of supplementary $MgCl_2$, increasing dNTP concentration, and varying template concentration.
- Based on the research detailed above, SSDE is a suitable medium for coral mucus swab samples, however results indicate that liquid nitrogen storage is preferable, resulting in increased quantities and higher amounts of PCR-quality DNA. If liquid nitrogen is unavailable, SSDE is an economical alternative storage medium, provided samples are processed in a timely manner once purified (i.e., sample DNA PCR-amplified within 24 h of DNA extraction).
- For analysis of any given set of study samples, it is recommended that the same preservation method be used for all coral mucus swabs.

References:

Concepcion, G. T., M. W. Crepeau, D. Wagner, S. E. Kahng, and R. J. Toonen. 2007. An alternative to ITS, a hypervariable, single-copy nuclear intron in corals, and its use in detecting cryptic species within the octocoral genus *Carijoa*. Coral Reefs **27**:323-336.

Dawson, M. N., K. A. Raskoff, and D. K. Jacobs. 1998. Field preservation of marine invertebrate tissue for DNA analysis. Molecular Marine Biology and Biotechnology **7**(2):145-152.

Ducklow, H.W., and R. Mitchell. 1979. Bacterial populations and adaptations in the mucus layers on living corals. Limnol. Oceanogr. **24**:715-725.

Harder, T., S. C. K. Lau, S. Dobretsov, T. K. Fang, and P. Qian. 2002. A distinctive epibiotic bacterial community on the soft coral *Dendronephthya* sp. and antibacterial activity of coral tissue extracts suggest a chemical mechanism against bacterial epibiosis. FEMS Microbiol. Ecol. **1464**:1-11.

Hansson, L., M. Agis, C. Maier, and M. G. Weinbauer. 2009. Community composition of bacteria associated with cold-water coral *Madrepora oculata*: within and between colony variability. Mar Ecol Prog Ser **397**:89-102.

Kellogg, C. A. 2004. Tropical Archaea: diversity associated with the surface microlayer of corals. Mar. Ecol. Prog. Ser. **273**:81-88.

Kellogg C. A., J. T. Lisle, and J. P. Galkiewicz. 2009. Culture-independent Characterization of Bacterial Communities Associated with the Cold-Water Coral *Lophelia pertusa* in the Northeastern Gulf of Mexico. Applied & Environmental Microbiology **75**:2294-2303

Lane, D. J., K. G. Field, G. J. Olsen, and N. R. Pace. 1988. Reverse transcriptase sequencing of ribosomal RNA for phylogenetic analysis. Meth. Enzymol. **167**:138–144.

Maidak B., N. Larsen, M. McCaughy, R. Overbeek., G. Olsen, K. Fogel, et al. 1996. The ribosomal database project. Nucleic Acids Res: **24**:82–85.

Rissanen, A. J., E. Kurhela, T. Aho, T. Oittinen, and M. Tiirola. 2010. Storage of environmental samples for guaranteeing nucleic acid yields for molecular microbiological studies. Appl. Microbiol. Biotechnol. **88**:977–984.

Ritchie, K. B. 2006. Regulation of microbial populations by coral surface mucus and mucus-associated bacteria. Marine Ecology-Progress Series **322**: 1-14

Rohwer, F., M. Breitbart, J. Jara, F. Azam, and N. Knowlton. 2001. Diversity of bacteria associated with the Caribbean coral *Montastraea franksii*. Coral Reefs **20**:85-91.

Rohwer F., V. Seguritan, F .Azam, and N. Knowlton. 2002. Diversity and distribution of coral associated bacteria. Marine Ecology Progress Series **243**:1-10

Rozman, D. and R. Komel. 1994. Isolation of genomic DNA from filamentous fungi with high glucan level. Biotech. **16**:382-384.

Sambrook, J., and D. Russell. 2001. Molecular Cloning: A Laboratory Manual (Third edition). Cold Spring Harbor Laboratory Press. Cold Spring Harbor, NY. 2,344 pp.

Sekar, R., D. K. Mills, E. R. Remily, J. D. Voss, and L. L. Richardson. 2006. Microbial communities in the surface mucopolysaccharide layer and the black band microbial mat of black band-diseased Siderastrea sidereal. Appl Environ Microbiol **72**(9):5963-5973.

Shaw, K. J., L. ThainPeter, T. Docker, C. E. Dyer, J. Greenman, G. M. Greenway, and S. J. Haswell. 2009. The use of carrier RNA to enhance DNA extraction from microfluidic-based silica monoliths. Analytica Chemica Acta **652:**231-233.

Woodley, C.M., A. W. Bruckner, A. L. McLenon, J. L. Higgins, S. B. Galloway and J. H. Nicholson. 2008. *Field Manual for Investigating Coral Disease Outbreaks*. NOAA Technical Memorandum NOS NCCOS 80 and CRCP 6. National Oceanic and Atmospheric Administration, Silver Spring, MD. 85pp.

Acknowledgements:

The authors wish to thank Dr. Christina Kellogg, St. Petersburg Coastal and Marine Science Center, USGS, for supplying the SSDE storage solution for the field samples and Dr. Caroline Rogers, Virgin Islands National Park, USGS, for performing the *Acropora palmata* mucus swabs. This project was funded in part by the NOAA Coral Reef Conservation Program.

This will be a blank page so that the back page will print correctly.

United States Department of Commerce
Gary F. Locke
Secretary

National Oceanic and Atmospheric Administration
Jane Lubchenco
Under Secretary of
Commerce for Oceans and Atmosphere,
NOAA Administrator

National Ocean Service
David Kennedy
Assistant Administrator