Comparison of DNA preservation methods for environmental bacterial community samples

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Abstract

Field collections of environmental samples, for example corals, for molecular microbial analyses present distinct challenges. The lack of laboratory facilities in remote locations is common, and preservation of microbial community DNA for later study is critical. A particular challenge is keeping samples frozen in transit. Five nucleic acid preservation methods that do not require cold storage were compared for effectiveness over time and ease of use. Mixed microbial communities of known composition were created and preserved by DNAgard™, RNAlater®, DMSO–EDTA–salt (DESS), FTA® cards, and FTA Elute® cards. Automated ribosomal intergenic spacer analysis and clone libraries were used to detect specific changes in the faux communities over weeks and months of storage. A previously known bias in FTA® cards that results in lower recovery of pure cultures of Gram-positive bacteria was also detected in mixed community samples. There appears to be a uniform bias across all five preservation methods against microorganisms with high G+C DNA. Overall, the liquid-based preservatives (DNAgard™, RNAlater®, and DESS) outperformed the card-based methods. No single liquid method clearly outperformed the others, leaving method choice to be based on experimental design, field facilities, shipping constraints, and allowable cost.

Introduction

In the past decade, there has been an increase in the number of coral-associated microbiology studies published. Many of these studies have used molecular techniques to examine microbial diversity, and as such, a common thread is the need to preserve the DNA of the bacterial community present in the coral holobiont. The often remote areas where corals thrive increase the necessity of preserving the bacterial community DNA for shipment and later processing. Field laboratory facilities, when present at all in these locations, are often extremely limited.

These limitations make immediate extraction and manipulation of coral-associated microbial DNA difficult to impossible. Some form of preservation is required. Currently, there are no standard protocols for the preservation of bacterial community DNA from coral samples. One of the methods for DNA preservation that has been very successful in many environments, flash-freezing in liquid nitrogen, has been used to preserve coral samples (Sunagawa et al., 2009), but liquid nitrogen can be difficult to obtain and transport, especially in the tropics. Preservation in ethanol has also been used, but its flammability significantly complicates shipping.

We therefore sought alternative DNA preservation methods that would minimize the need for laboratory equipment, refrigerators or freezers, and even electricity. Ideally, these methods should also be simple to use and make samples easy to ship (e.g. not dependant on hazardous materials like ethanol or dry ice). Five methods based on widely available technologies met these criteria and were chosen for evaluation. Table 1 contains a brief overview of the preservatives.

FTA® cards (Whatman, GE Healthcare, Little Chalfont, UK)

This method of DNA preservation involves the application of the sample onto a chemically treated filter card, which lyses the cells and preserves the DNA in the filter matrix. After air-drying, the card can be folded and stored at room temperature for years (Smith & Burgone,
A small punch of the card is then cleaned and used directly in the PCR. This technology has been used for the preservation of DNA from human buccal cells (Beckett et al., 2008), fish mucus (Livia et al., 2006), coral tissue and zooxanthellae (Crabbe, 2003), as well as pure cultures of bacteria (Lampel et al., 2000; Rajendram et al., 2006). The pure cultures of bacteria were not equally preserved, with Gram-positive cultures requiring an order of magnitude more colony-forming units (CFU) to be preserved than Gram-negative cultures (200–5000 CFU vs. 10–50 CFU) (Lampel et al., 2000; Rajendram et al., 2006). This indicates a bias because of differential cell lysis based on the chemistry of the cell wall.

**FTA Elute® cards (Whatman, GE Healthcare)**

Similar to the original FTA® card, the Elute® cards preserve the DNA in the filter matrix, but are designed so that the DNA is released into the eluent, rather than the card punch remaining in the PCR tube. This technology has been used for the preservation of human cells and viral detection (e.g. Gustavsson et al., 2009).

**RNAlater® (Ambion, Carlsbad, CA)**

A solution-based formula high in salts, RNAlater® preserves tissues for a week at room temperature, 1 month at 4 °C, and indefinitely at −20 °C. For long-term storage, fresh samples are immersed in the solution, refrigerated overnight to allow saturation of the tissue, and then frozen. RNAlater® has been shown to give better DNA yield than FTA® cards, with the added benefit of lower PCR inhibition (Nechvatal et al., 2008). RNAlater® is the only technology tested in this study that also provides reliable preservation of RNA for later analysis and can preserve samples for downstream histology (Florell et al., 2001).

**DNAgard™ (Biomatrica, San Diego, CA)**

DNAgard™, like RNAlater®, is designed to permeate the cells and preserve the DNA in a solution (Wilkinson et al., 2010). Unlike RNAlater®, this technology does not require refrigeration or freezing to maintain the stability of the DNA for long-term storage. Samples can be maintained at room temperature in liquid for 1 month, or the solution can be dried for extended storage.

**DMSO/EDTA/saturated sodium chloride (DESS)**

DESS is a nonproprietary solution that has been used to preserve DNA and has been shown to be superior to ethanol for coral tissue DNA preservation (Gaither et al., 2010). Like DNAgard™, samples in this solution can be stored long term (up to 24 weeks) at room temperature, though without the need for desiccation (Seutin et al., 1991), and this preservative has been used to preserve bacterial DNA from coral mucus swabs for up to 4.5 months (May et al., 2011).

The objective of this study was to compare the preservatives using a mixed bacterial community to uncover any differential preservation inherent in these methods. The comparison of these methods, including their ease of use in nonlaboratory conditions, will enable us to suggest criteria for the selection of an optimal preservative.

### Materials and methods

Two experiments were conducted: one long-term experiment using more Gram-negative species of bacteria to represent the microbial consortia frequently recovered from marine environments and a follow-up short-term experiment to better represent Gram-positive members of the microbial communities that may be present.

#### Experiment 1

Eight bacterial strains were selected from our culture collection to combine into a faux community. The strains were chosen to have a variety of characteristics that might impact preservation. The strains and selection criteria as well as culture collection numbers can be found in Table 2. The bacterial strains were grown in overnight culture and inoculated into fresh glycerol artificial seawater (Smith & Hayasaka, 1982) medium. Growth was monitored using a spectrophotometer to obtain estimates

### Table 1. Overview of products tested, their characteristics, and estimated cost

<table>
<thead>
<tr>
<th>Product</th>
<th>Medium</th>
<th>Room temperature stability</th>
<th>Long-term storage</th>
<th>Cost per sample (approx.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FTA®</td>
<td>Filter card</td>
<td>Years</td>
<td>Room temperature</td>
<td>$5/card</td>
</tr>
<tr>
<td>FTA Elute®</td>
<td>Filter card</td>
<td>Years</td>
<td>Room temperature</td>
<td>$6/card</td>
</tr>
<tr>
<td>DESS</td>
<td>Liquid</td>
<td>Months</td>
<td>Room temperature</td>
<td>$0.10/mL</td>
</tr>
<tr>
<td>RNAlater®</td>
<td>Liquid</td>
<td>1 week</td>
<td>Freezer (−20 °C)</td>
<td>$1/mL</td>
</tr>
<tr>
<td>DNAgard™</td>
<td>Liquid</td>
<td>Months</td>
<td>Room temperature, desiccated</td>
<td>$3/mL</td>
</tr>
</tbody>
</table>
of cell numbers, based on previous growth curves ground-truthed by plate counts of CFU (data not shown). When sufficient cell density had been reached, the cultures were diluted and mixed to obtain 10 mL of mixed culture with each strain represented at approximately $10^5$ CFU mL$^{-1}$. The individual cultures were also diluted and plated for confirmatory plate counts.

The DESS solution (0.25 M EDTA pH 7.5, 20% DMSO, and NaCl saturated) was prepared and autoclaved. All other preservatives were purchased ready-to-use from their manufacturers.

**Preservation**

Epicentre Catch-all™ swabs (Epicentre, Madison, WI) were used to sample the faux community by immersing the swab tip into the mixed culture liquid and rubbing the swab against the side of the culture tube to simulate sample collection. The swabs were processed based on the preservation method as follows, with three swabs preserved per preservation time interval (1 week, 1 month, 3 months, and 6 months) per preservative with five extra swabs per preservation method in case of accident. Three swabs were immediately extracted using the Qiagen DNeasy extraction kit (as described below for the RNAlater® and DNAgard™) to serve as an unpreserved control.

The swabs for the FTA® and FTA Elute® (Whatman, catalog numbers WB120311 and WB120401, respectively) preservation methods were pressed and rolled onto the FTA® cards until the card was thoroughly wetted with the culture. The cards were then air-dried in a biosafety cabinet 1–2 h and stored according to the manufacturer’s directions in a dark, dry place (a dark vacuum desiccator).

The RNAlater® (Ambion, catalog number AM7020), DNAgard™ (Biomatrica, catalog number 62001-036), and DESS swab tips were snipped off into 200 µL of solution using sterile scissors. The RNAlater® samples were stored overnight in the refrigerator at 4 °C and then frozen at −20 °C. The DNAgard™ samples were placed in a vacuum desiccator to dry and stored in the desiccator at room temperature (21 °C). The DESS samples were stored at room temperature in a box on the benchtop.

**Extraction and amplification**

For each time period, samples were extracted based on the preservation method as follows. Six 3-mm circular pieces were removed from the FTA® card-preserved samples with a sterilized punch and placed into six sterile PCR tubes. The card pieces were washed three times with 200 µL of FTA® Purification Reagent (Whatman) followed by two washes in TE$^{-1}$ (modified TE: 10 mM Tris–Cl, 0.1 mM EDTA, pH 8.0). The card pieces were then air-dried in a biosafety cabinet for 1 h. The amplification reaction was set up using the card pieces as templates.

FTA Elute® samples were extracted according to the manufacturer’s directions. Briefly, three 3-mm card pieces were removed from the card with a sterile punch. Each piece was placed in a microcentrifuge tube with 500 mL sterile DI water and vortexed three times for 1 s. The card pieces were transferred to new PCR tubes, and 30 µL of sterile DI water was added to each. The tubes were transferred to a heat block and incubated at 95 °C for 30 min. The eluate was removed to a new microcentrifuge tube, and 5 µL was used as the template for amplification.

The RNAlater®, DNAgard™, and DESS-preserved samples were extracted in triplicate using the Qiagen DNeasy kit (Qiagen, Valencia, CA). The preserved swabs were removed from the preservative and placed into the lysis buffer, as described by the Qiagen Gram-positive extraction protocol. The protocol was followed according to the manufacturer’s instructions, but the swabs were removed prior to the addition of ethanol (step 6).
Automated ribosomal intergenic spacer analysis (ARISA) amplification was carried out using the primers 16S-1392F (5'-GYACACACGCGCCGT-3') and 23S-125R [5(HEX)]-GGTTBCCCATTCRG-3' (Danovaro et al., 2006) and the following amplification conditions: 95 °C initial denaturation, 2 min; then 35 cycles of 95 °C, 1 min; 55 °C, 1 min; 72 °C, 2 min, followed by a 72 °C final extension, 10 min. Five microliters of template was used from each of the extraction methods, and the washed FTA® card punches remained in the PCR tubes during amplification. Each of the triplicate extraction samples was amplified in duplicate. Amplification was confirmed by gel electrophoresis, and positive duplicate amplifications were combined and cleaned using the Qiagen PCR cleanup kit (Qiagen). Positive amplifications were sent to the Roy J. Carver Biotechnology Center at the University of Illinois for fragment analysis via capillary electrophoresis. Additionally, DNA extractions from each of the individual bacteria were amplified and processed to have an ARISA profile for each strain to aid in deciphering the ARISA profiles of the mixed-assemble samples.

Fragment analysis trace data were analyzed using PeakScanner (Applied Biosystems, Carlsbad, CA), and the peak data were exported to R (www.r-project.org) for binning using the interactive binner script (Alban Ramette, Max Planck Institute for Marine Microbiology). Statistical analysis of binned data was performed using PRIMER 6 (PRIMER-E Ltd., Ivybridge, UK). Fragment results across triplicates were averaged, and square root transformed data were used to calculate Bray–Curtis similarities. Preservation methods were compared using ANOSIM and cluster analysis as described in Daniels et al., 2011.

Peak data were also manually called for the presence/absence of bacteria using the peak patterns from the individual bacteria and comparing those to the community profile returned from each sample. A sample was counted as positive if at least one of the characteristic peaks seen in the individual bacterium's pattern was present in two of the three replicate ARISA community profiles.

At the 6-month time period, a clone library was constructed from the liquid preservation methods using bacterial 16S primer 8F (Edwards et al., 1989) and universal primer 1492R (Stackebrandt & Liesack, 1993). Amplicons were cloned into the Qiagen PCR cloning plus kit (Qiagen) according to the manufacturer's instructions. From each of the libraries, 96 white colonies were picked and sent for sequencing to Northwoods DNA, Inc. (Bemidji, MN). Sequence data were compared to the GenBank database using BLAST (Altschul et al., 1990). Matches from the BLAST searches were used to identify bacterial strains still present in the preserved samples.

Experiment 1

Comparison of environmental DNA preservation methods (ARISA) showed that DNAgard®, DESS, and RNAlater® was strong, whereas FTA® and FTA Elute® samples amplified poorly (data not shown). ARISA profiles followed a similar pattern in total fluorescent signal (data not shown).

The cluster analysis comparing the various methods across all time frames (1 week to 6 months) is shown in Fig. 1. The analysis clusters all of the liquid-based preservation methods together with the time zero sample at > 60% similarity. Several of the card-based samples (the FTA® card samples from 1 week, 1 month, and 3 months, and the FTA Elute® 3 month) grouped separately at > 40% similarity.

ANOSIM comparison revealed statistically significant differences only with the following pairs: liquid-based (DNAgardTM, RNAlater®, and DESS) preservation vs. card-based (FTA® and FTA Elute®) preservation (R = 0.27, P = 0.01), DNAgardTM vs. FTA® (R = 0.5, P = 0.01), DESS vs. FTA® (R = 0.52, P = 0.01), and RNAlater® vs. FTA® (R = 0.51, P = 0.01).

The presence/absence data for the manually called peak analysis can be found in Table 3. All of the bacterial strains used in the study were amplified for ARISA individually to create an idealized community ARISA profile. See Fig. S1, Supporting Information for the peak traces of the individual bacteria used to make these identifications and a composite of those peaks into a faux community fingerprint. Mean preservation rates were calculated for preservation time period as well as for the preservation
method’s overall performance. DNAgard™ demonstrated the highest cumulative average preservation at 69%, followed by DESS (59%), RNAlater® (56%), FTA Elute® (50%), and FTA® (31%) (Table 3). *Citrobacter freundii* and the capsule-forming *Klebsiella pneumoniae* were recovered in every sample. *Aurantimonas coralicida*, the strain with the highest G + C content, was not recovered from any of the samples, including the control.

Clone library data reveal preservation percentages for DESS (77 clones) to be the highest (87.5%), while both RNAlater® (75 clones) and DNAgard™ (75 clones) preserved 75% of the bacterial strains. *Aurantimonas coralicida*, the strain with the highest G + C content, was not detected in any of the clone libraries, while RNAlater® and DNAgard™ both failed to retain *S. aureus*, the strain with the lowest G + C content and the only Gram positive. All other bacterial strains were recovered.

**Experiment 2**

PCR amplification showed similar results to the initial experiment, with the FTA® and FTA Elute® performing more poorly than the liquid-based methods. The cluster analysis comparing the various preservation methods across all time periods is shown in Fig. 2. As in the first experiment, the analysis clustered all of the liquid-based preservation methods together with the time zero sample at > 60% similarity. All of the FTA® samples and the FTA Elute® week three sample grouped separately from the time zero sample at > 40% similarity.

ANOSIM comparison echoed the initial experiment, indicating significant differences only between the liquid-based (DNAgard™, RNAlater®, and DESS) preservation vs. card-based (FTA® and FTA Elute®) preservation ($R = 0.78, P = 0.001$).

The presence/absence of the manually called peak data can be found in Table 4. The three bacteria that were substituted into the faux community in the second experiment were individually amplified using the ARISA primers to identify their primary peaks to use in the manual presence/absence analysis. Mean preservation rates were calculated for preservation time period as well as for the preservation method’s overall performance. DNAgard™ demonstrated the highest cumulative average preservation at 78%, followed by DESS (70%), RNAlater® (63%), FTA Elute® (44%), and FTA® (18%) (Table 4). Again, the *A. coralicida* was not recovered in any of the samples. *Citrobacter freundii* and *K. pneumoniae* were well preserved in most of the preparations, but this time not recovered from some of the card-based preparations. The other bacteria showed a similar pattern to the initial experiment. Of the new Gram-positive strains, *Bacillus subtilis* was recovered from all of the preservation methods. *Planomicrobius* sp. was recovered from all of the liquid-based
preservation methods and from the FTA Elute®-preserved samples from 1 and 2 weeks. Micrococcus sp. was only recovered in the unpreserved time zero sample.

**Discussion**

All of the analyses indicate the FTA® technology is the least suitable for the preservation of DNA from mixed microbial communities. With the most generous analysis method (the manually assessed presence/absence), the FTA® method never preserved more than 38% of the total community (Table 3) and fared even more poorly when the community contained more Gram-positive isolates (22%, Table 4). The ANOSIM comparisons showed a significant difference between the FTA® preservation method and each of the liquid-based methods, further suggesting that FTA® technology is more suited to its intended application (single DNA sources, like blood, tissue, and pure cultures) than for use with mixed microbial communities.

FTA Elute® technology performed better than the original FTA® card, but was outperformed by all of the liquid-based preservation techniques. In the best case scenario analysis, FTA Elute® preserved an overall average of 50% of the bacterial strains (Table 3). Over the long term, cluster analysis indicated variable performance from the technology, with the 1-month sample grouping with the T0 sample at < 40%, while the other time points grouped at > 60% (Fig. 1). The Bray-Curtis-based ANOSIM comparison of the FTA Elute® with the other preservation techniques did not reach statistical significance. The variability observed with the FTA Elute® technology is a likely factor in preventing statistical significance of the comparisons. However, further ANOSIM comparisons combining factors (card based vs. liquid based) did show a statistically significant difference in preservation. This indicates that, on the whole, card-based technologies are less reliable than the liquid-based methods for field use where preservation of a complex microbial community is required.

Cluster analysis grouped all of the liquid-based technologies together with the T0 sample at > 60% similarity, and there was less variation in their preservation abilities than was observed in the card-based technologies. The ANOSIM analyses did not find any significant differences when comparing DESS, DNAgard™, and RNAlater®. Cluster analysis does not allow us to rate one of these methods superior to another, and rating will have to be based on other criteria.

The best case scenario presence/absence results (Tables 3 and 4) do suggest some differences that the clustering analysis did not. Using this method averaged over all time periods, the DNAgard™ was the best performer followed by
DESS and then RNAlater®. The DESS data suggest good performance overall, but the 3-month sample performed poorly, preserving only 38% of the community; though, the other time periods are very similar to the DNAgardTM (Tables 3 and 4). Data from the clone library (Experiment 1, Table 5) are also very similar between the three liquid preservation methods, with DESS preserving the Gram-positive S. aureus’ DNA while the others did not.

Citrobacter freundii and K. pneumoniae are both Enterobacteriaceae, which can often have up to seven copies of the 16S rRNA gene, compared to, for example, α-proteobacteria that may often have as few as one (Ellwood & Nomura, 1980; Anderson & Roth, 1981). This may explain both their ease of recovery using all preservatives (Tables 3 and 4) and their high relative abundance in the clone libraries (Table 5).

The poor recovery of S. aureus in Experiment 1 was expected for the FTA® technologies because previous work had indicated a bias against preservation of Gram-positive organisms (Lampel et al., 2000; Rajendram et al.,

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**Table 4. Experiment 2**

<table>
<thead>
<tr>
<th></th>
<th>DNAgard</th>
<th>FTA Elute</th>
<th>FTA</th>
<th>RNA Later</th>
<th>DESS</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 W</td>
<td>2 W</td>
<td>3 W</td>
<td>1 W</td>
<td>2 W</td>
<td>3 W</td>
</tr>
<tr>
<td>A. coralicida</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>A. hydrophila</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>C. freundii</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Micrococcus sp.</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>P. hauseri</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Planomicrobium sp.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>S. marcescens</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Percent preserved</td>
<td>77.8</td>
<td>77.8</td>
<td>77.8</td>
<td>44.4</td>
<td>66.7</td>
<td>22.2</td>
</tr>
<tr>
<td>Average preservation</td>
<td>77.8</td>
<td>44.4</td>
<td>18.5</td>
<td>63</td>
<td>66.7</td>
<td>66.7</td>
</tr>
</tbody>
</table>

Manually called presence/absence of bacterial strains for each preservation method and time period (1 W = 1 week, 2 W = 2 weeks, 3 W = 3 weeks) and the initial T0 control. A ‘+’ indicates that the strain was present in at least one of the replicates.

**Fig. 2.** Cluster analysis of Experiment 2 (3 weeks, higher Gram-positive population) using square root transformed peak position and area data and Bray–Curtis similarity. Control = time zero sample with no preservation.
Table 5. Number of clones recovered from each of the clone libraries generated from the 6-month time period of Experiment 1 for each of the liquid preservation methods

<table>
<thead>
<tr>
<th></th>
<th>RNAlater®</th>
<th>DESS</th>
<th>DNAgar®™</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. coralicina</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>A. hydrophila</td>
<td>13 (17)</td>
<td>13 (17)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>C. freundii</td>
<td>26 (35)</td>
<td>34 (44)</td>
<td>40 (53)</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>13 (17)</td>
<td>4 (5)</td>
<td>4 (5)</td>
</tr>
<tr>
<td>P. hauseri</td>
<td>8 (11)</td>
<td>1 (1)</td>
<td>3 (4)</td>
</tr>
<tr>
<td>S. aureus</td>
<td>0 (0)</td>
<td>16 (21)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>S. marcescens</td>
<td>12 (16)</td>
<td>7 (9)</td>
<td>13 (17)</td>
</tr>
<tr>
<td>V. corallitucus</td>
<td>3 (4)</td>
<td>2 (3)</td>
<td>14 (19)</td>
</tr>
</tbody>
</table>

Percentage of the total number of clones is in parentheses. Percent of faux community preserved is the percentage of the eight strains present in the original faux community that were represented in the clone library.

2006); however, it was surprising to see in the other preservatives (Table 3). Even more unexpected was the total lack of recovery for A. coralicina. As we had no difficulties extracting and amplifying from this bacterium in isolation (Fig. S1), this cannot be blamed on either the extraction method or the specificity of the primers (PCR or ARISA). This result suggested that there might be an unrecognized bias in these preservation methods against high G + C organisms. Experiment 2 was run to better determine the recovery of both Gram-positive and high G + C bacterial DNA across all five methods. Two of the Gram-positive strains (Bacillus and Planimicrobium) were recovered 100% of the time in all three liquid preservatives and had variable recovery in the card-based methods (Table 4). This indicates that the liquid methods do not have a specific bias against recovery of Gram-positive organisms, while the FTA® technologies evidenced some. More telling was the fact that the two organisms with the highest percent G + C, A. coralicina (Gram negative) and Micrococcus (Gram positive), were not recovered by any preservation method (Table 4). This loss strongly suggests that there may be a bias in every preservation method surveyed against the recovery of high G + C organisms. This may explain some cases where A. coralicina, identified as the causative agent of white plague Type II, was not detectable in corals affected by this disease (e.g. Sunagawa et al., 2009).

The similarity of the performance of the liquid-based preservation methods suggests some flexibility in our choices. Considerations such as cost (Table 1), ability to be dried, or accessibility of refrigeration may then be taken into account. For highest performance, room temperature stability, and amenability to drying, DNAgard®™ would be the technology of choice; however, its relative lack of a track record in the literature and its cost may be more important factors to the researcher. The second best performer, DESS, is also the most cost-effective, because it is not proprietary and can easily be made in laboratories with fairly minimal resources; though, the variability as demonstrated in the 3-month sample suggests that it may not be a good long-term storage solution. Another issue with DESS that is not evident in swab samples is that the EDTA in it can leach calcium out of coral skeletons with prolonged storage, hindering later DNA recovery. The solution most widely used and with the largest literature footprint among coral microbiologists is RNAlater®, which performed well and with less variability than DESS. RNAlater® is also the only solution that will preserve both DNA and RNA in samples, as well as preserving tissues for histology. This makes it the most versatile of the solutions, but it is also the most limited because of its requirement for cold storage for periods longer than 1 week. Ethanol is a widely used preservative that was not evaluated in this experiment because of its requirement for cold storage and its flammability, both of which impede sample shipment. When tested against other methods, it has not been shown to be superior (e.g. Gaither et al., 2010).

The variability inherent in all of the community DNA preservation techniques reinforces the importance of sample replication. Increased replication may improve recovery of more members of the representative microbial community and correct partially for the variation that may occur with any preservation method. There is considerable variability in the microbial communities found associated with the coral holobiont. Both colony to colony of the same species and different locations on the same coral colony can host distinctly different microbial populations (Daniels et al., 2011). Many of the previous studies of coral microbial communities have been carried out with single samples, a method unlikely to generate a complete picture of the community (e.g. Sunagawa et al., 2009).

Despite starting with a roughly equal concentration of each of the bacteria in the faux community, the strains recovered in the clone libraries (Table 5) are not equally represented. This is likely due to several factors, including 16S rRNA gene copy number, differential preservation, differential extraction, and PCR amplification bias. This cannot likely be overcome with replication and shows relative abundance estimations based on 16S rRNA gene amplification of preserved samples should be interpreted with great care.

Initial ease of use is very similar with all of the liquid-based methods, that is, simply submerge the sample in the preservative. After the initial step, the procedures do differ slightly, and the choice of preservative would
depend on the facilities available. If there is no reliable electricity at the field site, for example, DESS or DNAgard™ would be preferred, or if shipment of liquids is to be avoided, DNAgard™ is the best option. The selection of the preservative ultimately depends on the experimental design, available facilities, and desired outcomes. There is, as yet, no perfect preservative for field-based studies.

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References


Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Individual ARISA profiles generated for each 1 of the bacterial strains in the Experiment 1 faux community and an idealized composite community profile. These profiles were used for the manually-called presence/absence analysis in Table 3. Similar individual profiles were generated for the faux community in Experiment 2 in order to produce Table 4 (data not shown).

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