MICROBIAL BIOMARKERS FOR ASR-DAMAGED CONCRETE

Date: February 2018

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Prepared for:
Virginia Center for Transportation Innovation and Research
530 Edgemont Road
Charlottesville, VA 22903
2. Government Accession No.  
3. Recipient’s Catalog No.  

4. Title and Subtitle  
MICROBIAL BIOMARKERS FOR ASR-DAMAGED CONCRETE  

5. Report Date  
4/30/2018  

6. Performing Organization Code  

7. Author(s)  
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10. Work Unit No. (TRAIS)  

11. Contract or Grant No.  
DTRT13-G-UTC33  

12. Sponsoring Agency Name and Address  
US Department of Transportation  
Office of the Secretary-Research  
UTC Program, RDT-30  
1200 New Jersey Ave., SE  
Washington, DC 20590  

13. Type of Report and Period Covered  
Final 7/1/15 –2/28/18  


15. Supplementary Notes  

16. Abstract  
Concrete is the most widely used building material in the world, and deterioration of concrete infra-structure is likewise a global problem. Current methods for identification of damage rely heavily on hu-man observation, forcing departments of transportation as well as other agencies to replace structures after damage has already occurred. Bio-indicators, primarily bacteria associated with specific risk factors for disease, have long been used to identify risk in food production, water treatment, and recreational waters. Here, we proposed the identification of bacteria that could serve as bio-indicators for early stages of alkali-silica reaction (ASR), a common mechanism contributing to concrete deterioration in the Mid-Atlantic region. Because the chemistry of ASR-affected concrete differs from that of undamaged concrete, we predicted that the microbial population in ASR-affected concrete would also be different. Identification of bioindicators for early detection of ASR could allow state and local departments of transportation to treat ASR, or provide them with more time to budget and plan the replacement of affected structures.  

This work had two phases: Phase I included proof-of-principle experiments demonstrating that viable bacteria were present in concrete, and that DNA could be extracted from concrete and used to comprehensively identify the bacteria in concrete. Phase II was a 2-year time series analyzing DNA in ASR-reactive and ASR-mitigated concrete samples. The samples weathered outside and were collected every 4-8 weeks over the course of the series. DNA was extracted from all samples and analyzed to monitor changes in the concrete “microbiome” over time. This work demonstrated that viable bacteria inhabit ordinary concrete and that DNA can be easily extracted from small (~5-g) samples. We further showed that these bacteria primarily come from the large aggregate and that some of the bacteria in ASR-affected concrete are different from those in unaffected concrete. Organisms in the Staphylococcus, Aeromicrobium, Chitinophaga, Sediminibacterium, and Xenococcus genera may be useful bio-indicators, and future work will examine these in more detail. This method can potentially be applied to other types of chemical damage affecting concrete.  

17. Key Words  
Concrete, alkali-silica reaction, bio-indicators, bacteria, DNA  

18. Distribution Statement  
No restrictions. This document is available from the National Technical Information Service, Springfield, VA 22161  

19. Security Classif. (of this report)  
Unclassified  

20. Security Classif. (of this page)  
Unclassified  

21. No. of Pages  
XX  

22. Price  

EXECUTIVE SUMMARY

Concrete is the most widely used building material in the world, and deterioration of concrete infrastructure is therefore a global problem. Current methods for identification of damage concrete rely heavily on human observation of visible damage, which is often only apparent after extensive internal damage has occurred. This often gives departments of transportation and other agencies very short time frames for planning, repair, or replacement of affected structures. Bio-indicators, primarily bacteria associated with specific risk factors for disease, have long been used to identify risk in food production, water treatment, and recreational waters. Here, we proposed the identification of bacteria that could serve as bio-indicators for early stages of alkali-silica reaction (ASR), a common mechanism contributing to concrete deterioration in the Mid-Atlantic region. Because the chemistry of ASR-affected concrete differs from that of undamaged concrete, we predicted that the microbial population in ASR-affected concrete would also be different. Identification of bio-indicators for early detection of ASR could allow state and local departments of transportation to treat ASR, or provide them with more time to budget and plan the replacement of affected structures.

This work had two phases: Phase I included proof-of-principle experiments demonstrating that viable bacteria were present in concrete, and that DNA could be extracted from concrete and used to comprehensively identify the bacteria in concrete. Phase II was a 2-year time series analyzing DNA in ASR-reactive and ASR-mitigated concrete samples. The samples weathered outside and were collected every 4-8 weeks over the course of the series. DNA was extracted from all samples and analyzed to monitor changes in the concrete “microbiome” over time. This work demonstrated that viable bacteria inhabit ordinary concrete and that DNA can be easily extracted from small (~5-g) samples. We further showed that these bacteria primarily come from the large aggregate and that some of the bacteria in ASR-affected concrete are different from those in unaffected concrete. Organisms in the Staphylococcus, Aeromicrobium, Chitinophaga, Sediminibacterium, and Xenococcus genera may be useful bio-indicators, and future work will examine these in more detail. This method can potentially be applied to other types of chemical damage affecting concrete.
Acknowledgments:

This project was funded by the Mid-Atlantic Transportation Sustainability Center – Region 3 University Transportation Center (MATS UTC). Figures 4 and 5 and Table 3 are reproduced with permission from (Maresca et al., 2017).

We gratefully acknowledge colleagues at the Delaware Department of Transportation, especially David Dodd, for providing the materials for the Phase II concrete samples. Dr. Ardeshir Faghri and Dr. Thomas Schumacher at the University of Delaware were instrumental in the early stages of this project. We thank Gary Wenczel, in the UD Structures Laboratory, for his assistance in mixing and pouring the concrete cylinders. Dr. Jessica Keffer helped to develop the DNA extraction procedure, validated it, and did the initial sequence analysis. Anders Kiledal did the majority of the analysis presented in Figs. 6-12. Several undergraduate researchers, including Mary Katherine Sutter, Paul Moser, and Alison Treglia, also contributed to this work.

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1.1 Problem

Concrete is the most widely used building material in the world (Chen and Liew, 2003). Although concrete can be long-lasting – the Romans used concrete to build bridge piers, jetties, and even the dome of the Pantheon nearly 2,000 years ago (Delatte, 2001) – most concrete structures are built with a finite predicted service lifetime of ~100 years (Wegen et al., 2012). As these structures age, detection of damage or potential damage becomes important for both planned use and planning for replacement or repair. Currently, structural health is assessed using primarily visual methods, which can be subjective (Arndt et al., 2011). For this reason, there is a serious need for simple, minimally-invasive diagnostic tests that can identify damage prior to visible deterioration.

In food, health, and water infrastructure, specific kinds of bacteria or other microbes associated with specific problems are used as “bioindicators” to identify risks of disease or contamination that would otherwise be undetectable (Amini and Kraatz, 2014; Marine et al., 2015). Based on the routine use of bioindicators in other systems, we believe that they may also be applicable to evaluation of concrete infrastructure as well. Deterioration mechanisms such as alkali-silica reaction (ASR), carbonation, or corrosion of reinforcing steel cause chemical changes in the concrete that alter the environment inhabited by microbes. These chemical changes could potentially lead to observable changes in the assemblage of microbes in concrete, making it more hospitable to some microbes and less hospitable to others. For this reason, the presence of particular species of bacteria may indicate the physical and chemical conditions in concrete that could lead to structural damage. However, bacteria known to be associated with concrete damage are associated with biofilms on concrete surfaces, not internal to concrete structures (reviewed in Wei et al., 2010). Environmental conditions on the surface of concrete can be quite different from the internal conditions, and different bacteria would be expected to live inside the concrete.

Therefore, to assess the potential for use of bioindicators in structural evaluation, we must first confirm that viable bacteria are present in concrete, then develop a method for identifying these bacteria. Some bioindicator tests, such as enumeration of fecal coliforms in recreational or drinking waters, require cultivation of bacteria in the laboratory. Because the bio-indicator microbes in these tests may be patchily distributed, present in very low concentrations, or even unrelated to the risk assessed, cultivation-based assays are not currently regarded as the best practice (Harwood et al., 2014). More recently, methods have
been developed that detect genes unique to bioindicator species in the environment or sample being evaluated (Harwood et al., 2014). These methods are both more sensitive and more robust than culture-based tests, but require extraction of high-quality DNA from the sample. The goals of this research were thus to (1) perform proof-of-principle experiments to show that bacteria in and on concrete could be detected and analyzed, then (2) investigate the potential for identification and application of bioindicators to detect ASR damage in concrete.

ASR is a chemical reaction between the alkaline cementitious materials and reactive silica present in certain aggregates, and is a common mechanism contributing to concrete deterioration in the Mid-Atlantic (Touma et al., 2001). When there are available cations in the concrete pore solution, reactive silica in the aggregate, and sufficient moisture, a gel-like material forms in the concrete, absorbs water and expands, exerting tensile forces within the concrete matrix and causing cracking (Fig. 1). External water easily penetrates the cracked concrete, exacerbating ASR and increasing the potential for other kinds of damage, further deteriorating the concrete. Because the chemistry of ASR-affected concrete differs from that of undamaged concrete, we predicted that its microbial population would also be different. Identification of bioindicators for early detection of ASR could allow state and local departments of transportation to treat ASR, or provide them with more time to budget and plan the replacement of affected structures.

Here, we demonstrate that viable bacteria inhabit ordinary concrete and that DNA can be easily extracted from small (~5-g) samples. We further show that these bacteria primarily come from the large aggregate, that some of the bacteria in ASR-affected concrete are different from those in unaffected concrete, and that *Staphylococcus*, *Aeromicrobium*, *Chitinophaga*, *Sediminibacterium*, and *Xenococcus* are potentially useful bio-indicators.

1.2 APPROACH

In order to demonstrate that bacteria in concrete are alive and can be analyzed, we first cultivated some of the viable bacteria in concrete (Fig. 2), then developed a method for extraction of DNA from concrete. After analysis of this DNA confirmed that this approach would be feasible, we prepared 15 small test cylinders using concrete materials from the Delaware Department of Transportation (DelDOT) known to be highly susceptible to ASR, and a parallel series of 15 test cylinders that incorporated DelDOT’s standard mitigation against ASR. All cylinders were placed outside on a roof to weather, and one cylinder from each series was removed from the roof every 6-8 weeks and archived. DNA was extracted from three sub-samples of each cylinder, bacterial 16S genes were amplified and sequenced using the Earth Microbiome Project protocol (Gilbert et al., 2014; Thompson et al., 2017), and sequences were analyzed using Qiime2 software (https://qiime2.org/).
2. METHODOLOGY

2.1 PROOF-OF-PRINCIPLE EXPERIMENTS

2.1.1 Production of concrete cylinders for proof-of-principle experiments

To determine whether ordinary concrete is colonized by microbes, concrete cylinders (152 × 305 mm) were cast in December 2011 using a Delaware Department of Transportation (DelDOT) Class B mix design (Table 1), which is typically used for concrete pavements. Crushed coarse aggregates had a maximum size of 19 mm.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Design compressive strength, fc'</td>
<td>20 MPa (2900 psi)</td>
</tr>
<tr>
<td>Design cement content (minimum)</td>
<td>334 kg/m³ (560 lb/yd³)</td>
</tr>
<tr>
<td>Design water-to-cement ratio, w/c</td>
<td>0.45</td>
</tr>
<tr>
<td>Required air content</td>
<td>4–7 %</td>
</tr>
<tr>
<td>Required slump</td>
<td>50–100 mm (2–4 in.)</td>
</tr>
<tr>
<td>Required admixtures</td>
<td>D, E, F, G</td>
</tr>
</tbody>
</table>

TABLE 1. DELDOT CLASS B MIX DESIGN. Specifications for the concrete used for proof-of-principle experiments were obtained from the Delaware Department of Transportation.

After casting, the cylinders were cured in capped plastic forms for 2 weeks, then uncapped and kept in a storage room at room temperature until February 2012, when two cylinders were placed on the roof of a University building in Newark, Delaware, where they were exposed to weather. After 12 months of exposure, samples for cultivation and DNA analysis were collected.

2.1.2 Cultivation of viable bacteria from concrete.

Prior to sampling, cylinders were moved from the rooftop to a pre-sterilized sampling area. All tools were sterilized with 95 % ethanol. The top surface of one cylinder was struck using a 3-in. masonry chisel and hammer until surface chips or larger chunks spalled off. Individual chips were transferred to sterile buffer (10 mM Tris, 1 mM EDTA) in 50-mL centrifuge tubes. The samples were vortexed for 10 s and 25 μL of the buffer was spread on Petri dishes filled with solid CM-A medium, a minimal, slightly alkaline medium developed for this study. CM-A contains (per liter), 1 g NaHCO₃, 1.44 g ammonium acetate, 0.12 g

![Figure 2: Cultivation Strategy. Concrete chips were dropped into sterile saline solution and mixed, then the solution was spread onto growth medium and incubated until colonies appeared. Individual colonies were then restreaked on fresh medium until only one kind of bacteria was present.]
MgSO$_4$·7H$_2$O, 0.14 g KH$_2$PO$_4$, 0.17 g K$_2$HPO$_4$, 1 mL each Wolfe’s vitamin and trace minerals solutions (Wolin et al., 1963), and 11 mL phosphate buffer (per liter, 62.7 g KH$_2$PO$_4$ and 1.27 g K$_2$HPO$_4$, pH 8.5). The pH of the medium was adjusted to 8.0. The Petri dishes were incubated at room temperature (~23°C) for 2 weeks. Individual colonies were restreaked onto fresh CM-A media until only one colony type was visible. Isolation of bacterial strains was verified by microscopy and 16S rRNA sequencing. Once isolated, strains were archived at −80 °C in 10 % glycerol.

2.1.3 Development of DNA extraction method

Subsamples of the concrete cylinders were obtained first by breaking off pieces of the concrete as described in section 2.2, then grinding ~5 g samples to powder in a mortar and pestle.

To facilitate comparison of the sequence data to data obtained from other environments, we followed the recommendations of the Earth Microbiome Project to the degree possible (http://www.earthmicrobiome.org/). For this reason, DNA extraction from concrete was initially attempted using the MoBio PowerSoil and PowerMax DNA extraction kits (MoBio, Carlsbad, CA, catalog nos. 12888 and 12988, respectively), but no genomic DNA was obtained. We then attempted a method developed for DNA extraction from soil, with a high-salt, high-temperature lysis step (Zhou et al., 1996), but again no DNA was obtained.

Concrete is dry, hard, and high in divalent cations such as Ca$^{2+}$. Other materials with similar characteristics include limestone, mineral-rich sediments, and dehydrated bone. A method used for extraction of DNA from endolithic communities was also attempted (Hugenholtz et al., 1998), but no DNA was obtained using this method. Another method developed for extraction of nucleic acids from high-silica sediments, with an alkaline incubation at high temperature to dissolve the silicate minerals (Kouduka et al., 2012), was also attempted without success.

Subsequently, methods developed for extraction of DNA from ancient bone were modified for application to concrete samples (Cattaneo et al., 1995; Loreille et al., 2007). In these methods, the sample is washed with EDTA (ethylenediaminetetraacetic acid) prior to extraction, to remove some of the divalent cations, then additional salts are precipitated using a sodium acetate solution. In the method we developed for concrete, each 5-g sample of crushed concrete was washed with 20 mL of 0.5 M EDTA. Because so many of the bacteria we isolated were Gram-positive bacteria with membranes that can be difficult to disrupt, we also included two enzymatic lysis steps. The EDTA-washed powder was suspended in 15 mL of lysis buffer (40 mM EDTA, 50 mM Tris pH 8.3, 0.73 M sucrose) with 15 mg of lysozyme per gram of concrete powder and incubated at 37 °C with gentle agitation for 30 min to break open bacterial cell walls. Next, 100 µL of proteinase K (20 mg mL$^{-1}$) and 1.5 mL of 20 % sodium dodecyl sulfate (SDS) were added and the solution was incubated at 56 °C for 2.5–3 h.
with gentle agitation to break any remaining cell walls. Membranes, proteins and cell debris were separated from DNA and other water-soluble material by extraction with 20 mL chloroform. To remove water-soluble non-nucleic acid material, 1 mL of 1.95 M sodium acetate was added to the aqueous phase of each sample after extraction and mixed vigorously for 30 s. The sample was then centrifuged and the aqueous phase was transferred to a fresh centrifuge tube and re-extracted with 0.8 volume of chloroform. DNA was precipitated with 1 volume of isopropanol and 0.1 volume 3 M sodium acetate. The DNA pellet was washed with 70% ethanol, and genomic DNA was then re-suspended in 25 µL of sterile water and stored at −20 °C. This method has been published in *Materials and Structures* (Maresca et al., 2017).

Using this protocol, we consistently obtain high-molecular-weight genomic DNA from concrete (Fig. 3A). This DNA is pure enough for downstream processing by polymerase chain reaction (PCR) amplification and sequencing (Fig. 3B). To confirm that biomass is not lost during the EDTA wash, DNA was extracted from the EDTA supernatant after the wash step. No genomic DNA was observed in this fraction, confirming that the additional wash steps do not result in appreciable loss of biomass or DNA from the samples.

### 2.1.4 DNA sequence analysis (Phase I)

The extracted genomic DNA was sent to Research and Testing Laboratories (Lubbock, TX) for 16S rRNA gene amplification and sequencing. The 16S gene in bacteria is used for species identification. For general analysis of all organisms in an environment, primers that amplify a region of this gene that is found in all species are used, then the genes are sequenced to identify the organisms present. For more specific analysis such as presence/absence of bioindicator species, different primer sets unique to the organisms of interest can be designed. Here, general primers for amplification of the V3–V4 region of the gene were used (515F and 806R (Caporaso et al., 2012)). Sequencing was performed on an Illumina MiSeq instrument; both amplification and sequencing were done by Research and Testing Laboratories (Lubbock, TX).

Paired raw reads were joined, quality-filtered and assigned to taxonomic groups using Qiime1 pipelines (Kuczynski et al., 2012; Aronesty, 2013). Operational taxonomic units (OTUs, analogous to species) were defined as gene clusters sharing 97% sequence identity. Alpha and beta diversity parameters were calculated using standard Qiime pipelines (Kuczynski et al., 2012).

### 2.2 ASR TEST SERIES

#### 2.2.1 Preparation of ASR-prone and ASR-mitigated concrete test cylinders

Materials for ASR-reactive concrete were obtained from the Delaware Department of Transportation (DelDOT). Two parallel batches of concrete were mixed in the UD Structures Laboratory. The ASR-reactive mix, with no mitigation to prevent ASR, used the mix design for DelDOT Class A-503 concrete (Table 2). The ASR-mitigated mix, which used the same materials but replaced half of the cement with slag, used the mix design for DelDot Class A-3-50 (Table 2). The batches were initially mixed according to the specifications, but some additional water was added to improve workability prior to pouring.
Concrete was poured into 4”d x 8”h cylindrical plastic forms, which were agitated to remove air pockets, then capped and placed in a storage room to cure for 2 weeks. After curing, cylinders were removed from the forms and placed on the roof of Colburn Laboratory on the University of Delaware campus, where they would be exposed to wind, sun, and precipitation, but protected from foot and car traffic. Two cylinders (one from each series) were archived immediately (May 2, 2013) without being placed on the roof. After this, one cylinder from each series was archived approximately every 4-8 weeks until February 17, 2015.

<table>
<thead>
<tr>
<th>Component*</th>
<th>Per yd³</th>
<th>Batch</th>
<th>Per yd³</th>
<th>Batch</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASR-reactive DelDOT Class A-503</td>
<td>Non-reactive (mitigated) DelDot Class A-3-50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cement</td>
<td>799.0</td>
<td>44.4</td>
<td>353.0</td>
<td>33.9</td>
</tr>
<tr>
<td>Slag</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Silica Fume</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Water</td>
<td>267.7</td>
<td>14.9</td>
<td>282.0</td>
<td>27.0</td>
</tr>
<tr>
<td>Air</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Fine Aggregates</td>
<td>1070.0</td>
<td>59.4</td>
<td>1064.0</td>
<td>102.1</td>
</tr>
<tr>
<td>Coarse Aggregates</td>
<td>1881.0</td>
<td>104.5</td>
<td>1870.0</td>
<td>179.4</td>
</tr>
<tr>
<td>Admix 1</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Admix 2</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Total weight</td>
<td>4017.7</td>
<td>223.2</td>
<td>3922.0</td>
<td>217.9</td>
</tr>
<tr>
<td>w/c Ratio</td>
<td>0.335</td>
<td>0.34</td>
<td>0.399</td>
<td>0.40</td>
</tr>
</tbody>
</table>

TABLE 2. Mix designs for ASR-reactive (class A-503) and ASR-mitigated concrete (class A-3-50). Mix designs and materials were obtained from DelDOT.

*All units are pounds.

2.2.2 DNA extraction and sequencing from time series

Subsamples (5 g) were collected from 3 different locations on each cylinder with a drill core (3/4” inch bit) sterilized with ethanol. Each subsample was then crushed to powder in a rock grinder and stored at -80°C until processing. After crushing, DNA was extracted as described in section 2.1.3.

DNA was also extracted from the materials used in the concrete mixes. The same procedure was used to extract DNA from large aggregate, fine aggregate, Portland cement, and slag. To identify any contaminants that might be introduced during either the extraction process or the amplification and sequencing processes, glass beads (5 g) were sterilized by bleaching, UV irradiation, and autoclaving, then processed as the concrete and materials were.

2.2.3 DNA sequence analysis and identification of putative bioindicators

The V3-V4 region of the 16S genes in all samples was amplified using primers 357F and 806R at the UD Sequencing and Genotyping Center. Samples were then sequenced using an Illumina MiSeq system, with paired 301-base-pair reads. Read pairs were quality-filtered and joined, then grouped into amplicon sequence variants (ASVs, equivalent to species), and assigned to specific taxonomic groups using standard parameters in Qiime2 (https://qiime2.org/).

3 FINDINGS

3.1 PHASE I: PROOF-OF-PRINCIPLE EXPERIMENTS
To demonstrate that bacteria in concrete are alive and can be analyzed, we first cultivated some of the bacteria in concrete (Fig. 2), then developed a method for extraction of DNA from concrete. Analysis of this DNA confirmed that a DNA-based approach to analysis of bacterial assemblages in and on concrete would be feasible.

### 3.1.1 Culture collection

Fifteen bacterial strains were isolated from the surface and inside of the concrete samples. Greater diversity was initially observed on solid media spread with samples from surfaces than interior samples. Individual colonies were transferred to fresh solid medium until the culture contained only one kind of organism, as assessed by microscopy and 16S rRNA amplification and sequencing.

Nearly all of the bacteria isolated from the concrete test samples are Actinobacteria, primarily *Arthrobacter* spp. (Table 3). One representative each of Alphaproteobacteria (*Paracoccus* species) and Gammaproteobacteria (*Pseudoalteromonas*) were also isolated. Most of these strains are brightly colored due to the synthesis of carotenoid pigments (data not shown), and grow readily on both solid and liquid medium using acetate as the carbon and energy source. Relatives of several of these species were isolated from environments that are similar to concrete in salinity or alkalinity. For example, *Kocuria* species have been found in marine sediments (Kim et al., 2004) and salt evaporation works (Sarafin et al., 2014). *Dietzia natronolimnaea* strains have been isolated from moderately saline alkaline lakes (Duckworth et al., 1998), and *Nocardioides lentus* has been isolated from alkaline soils (Yoon et al., 2006).

<table>
<thead>
<tr>
<th>Strain designation</th>
<th>Phylum</th>
<th>Closest relative in GenBank</th>
<th>Colony color</th>
<th>Cell Shape</th>
</tr>
</thead>
<tbody>
<tr>
<td>IN-02CB</td>
<td>Alphaproteobacteria</td>
<td><em>Paracoccus marinus KKL-B9</em></td>
<td>Green-Yellow</td>
<td></td>
</tr>
<tr>
<td>IN-02</td>
<td>Gammaproteobacteria</td>
<td><em>Pseudoalteromonas</em> sp. BSI20669</td>
<td>Orange</td>
<td>short rod</td>
</tr>
<tr>
<td>IN-01</td>
<td>Actinobacteria</td>
<td><em>Arthrobacter</em> sp. 32c</td>
<td>White</td>
<td>short rod</td>
</tr>
<tr>
<td>IN-02C</td>
<td>Actinobacteria</td>
<td><em>Kocuria marina</em></td>
<td>Green-Yellow</td>
<td>coccus</td>
</tr>
<tr>
<td>IN-03</td>
<td>Actinobacteria</td>
<td><em>Dietzia cercidiphylli</em> strain X10</td>
<td>Orange</td>
<td>rod</td>
</tr>
<tr>
<td>IN-04</td>
<td>Actinobacteria</td>
<td><em>Rhodococcus cercidiphylli</em> strain EB37</td>
<td>Orange-Yel-</td>
<td>long rod</td>
</tr>
<tr>
<td>IN-06</td>
<td>Actinobacteria</td>
<td><em>Arthrobacter</em> sp. clone EK_Ca790</td>
<td>Pink</td>
<td>rod</td>
</tr>
<tr>
<td>IN-06B</td>
<td>Actinobacteria</td>
<td><em>Kocuria marina</em></td>
<td>Yellow-Green</td>
<td>coccus (pairs)</td>
</tr>
<tr>
<td>IN-07</td>
<td>Actinobacteria</td>
<td><em>Arthrobacter agilis</em> strain DSM 20550</td>
<td>Pink</td>
<td>coccus</td>
</tr>
<tr>
<td>IN-08</td>
<td>Actinobacteria</td>
<td><em>Nocardioides lentus</em> strain KSL-19</td>
<td>Burnt Orange</td>
<td></td>
</tr>
<tr>
<td>IN-09</td>
<td>Actinobacteria</td>
<td><em>Dietzia natronolimnaea</em> strain NF047</td>
<td>Orange-Red</td>
<td>rod</td>
</tr>
<tr>
<td>CL-01</td>
<td>Actinobacteria</td>
<td><em>Dietzia natronolimnaea</em> strain NF047</td>
<td>Orange</td>
<td>short rod</td>
</tr>
<tr>
<td>CL-02</td>
<td>Actinobacteria</td>
<td>Uncultured <em>Arthrobacter</em> sp. clone Acro223</td>
<td>Yellow-Green</td>
<td>coccus</td>
</tr>
<tr>
<td>CL-04</td>
<td>Actinobacteria</td>
<td><em>Arthrobacter</em> sp. 5139</td>
<td>White</td>
<td>coccus (pairs)</td>
</tr>
<tr>
<td>CL-05</td>
<td>Actinobacteria</td>
<td><em>Rhodococcus erythropolis</em> strain zxx26</td>
<td>Yellow-Green</td>
<td>coccus</td>
</tr>
</tbody>
</table>

**TABLE 3. BACTERIA ISOLATED FROM CONCRETE.** All strains were identified by amplification of the 16s rRNA gene with primers 8F and 1492R and Sanger sequencing of the products. Strains designated “CL” were isolated from top or side surfaces of concrete; strains designated “IN” were isolated from internal samples. They were compared to GenBank, the non-redundant gene database maintained at the National Library of Medicine (ncbi.nlm.nih.gov), and the closest cultivated relative of each of our isolates was identified in this database. This table is reprinted with permission from Maresca, Moser and Schumacher, 2017.
Isolation of these bacteria demonstrated that there are bacteria in and on concrete, and that at least some of these bacteria are alive. One concern, prior to this analysis, had been that any bacteria that were incorporated into concrete from the materials would be killed either by the extreme conditions in the concrete (at >12, the pH in concrete is nearly the same as the pH of bleach, which is used for disinfection) or by the high temperature during curing. DNA is, in fact, quite stable, and it is possible that DNA found in concrete could have been “relic DNA” (Carini et al., 2016). This is still a possibility. However, the presence of viable bacteria in concrete demonstrates that at least some of the bacteria in and on concrete are not just alive but may respond to changing conditions.

3.1.2 Proof-of-principle: DNA analysis of test sample

We sequenced only two samples (DNA extracted from a surface subsample, and DNA extracted from an interior subsample) in this proof-of-principle experiment, and obtained ~25,000 DNA read pairs from DNA extracted from the top of the concrete cylinder, and ~59,000 read pairs from the inside sample. Since each 16S gene was sequenced from both ends, this number of reads meant that approximately 24,000 16S genes from the top sample and 49,000 from the inside sample were completely sequenced. More than 250 bacterial genera were identified in these sequence data sets. The most abundant groups in both the interior and surface samples were Actinobacteria and Proteobacteria (Fig. 4). The Micrococcaceae, a subset of Actinobacteria that includes the Arthrobacter spp. and Nocardioidaceae that we isolated, were abundant in the interior sample. These organisms, though they do not form spores, are capable of forming dormant cyst-like cell types when conditions are unfavorable to growth (Demkina et al., 2000; Young et al., 2010; Shimkets, 2013). Approximately 2.5% of the sequences in the interior sample belong to the Firmicutes (Bacillus and Clostridium species), which do form spores; in total, Gram-positive bacteria (Actinobacteria and Firmicutes) comprise more than 65% of the species identified in the interior sample (Fig. 4A). Of the Proteobacteria in the interior sample, the most abundant were Alphaproteobacteria, specifically Rhizobia and Caulobacteria, which are both common soil bacteria. Nearly 6% of the sequences in this sample could not be assigned to specific taxa, likely because they were equally similar to multiple types of bacteria.

In contrast, the microbial assemblage on the surface of the cylinder was only ~33% Actinobacteria (Fig. 4B). In this sample, the Alphaproteobacteria and Betaproteobacteria species were much greater fractions of the population (Fig. 5B). Unsurprisingly, the surface sample also had more sequences derived from photosynthetic Cyanobacteria (blue-green algae) and chloroplasts (plant-associated organelles that likely came from pollen in these samples) than the interior sample. Spore-forming Firmicutes were an even smaller fraction of the surface sample than in the interior sample. Deltaproteobacteria related to Bdellovibrio and Myxococcus, predatory species that consume other bacteria, were observed only in the top sample (Fig. 4B).

The DNA sequence analysis demonstrated that the microbial assemblages had much greater diversity than observed in the culture collection. This discrepancy between cultivation-dependent and cultivation-independent analyses of the same environment is common, since we know very little about the nutritional requirements of environmental bacteria (Epstein, 2013; Giannantonio, Kurth, Kurtis, & Sobecky,
A. Geodermatophilaceae (Moser and Schumacher, 2017) successfully cultivated in our laboratory (see Table 3). This figure is reprinted with permission from Maresca, and the names are family-level identification. Asterisks (*) indicate that organisms from this group were successfully cultivated in our laboratory (see Table 3). This figure is reprinted with permission from Maresca, Moser and Schumacher, 2017.

B. Microbes found on the surface of the concrete sample, identified by 16s rRNA gene sequencing. In both panels, colors indicate phylum-level identification, and the names are family-level identification. Asterisks (*) indicate that organisms from this group were successfully cultivated in our laboratory (see Table 3). This figure is reprinted with permission from Maresca, Moser and Schumacher, 2017.
All of the actinobacterial species that we isolated belong to genera identified in the sequence data set (see the taxa indicated with * in Fig. 4). However, the species successfully cultivated are not the most numerically abundant in the sequence data set. For example, although six of our 13 actinobacterial isolates belong to the Corynebacteria, they represent less than 0.3% of the sequences from the interior sample, and an even smaller fraction of the surface sample. Micrococcales are well-represented in both the sequence data sets and in our collection of isolates. Better understanding how these isolates survive and grow in concrete will therefore give us information about representative bacteria in this unique environment.

The gene amplification step of this analysis has some biases, so it cannot capture 100% of the total bacterial diversity. However, we can use statistical analyses to determine whether our analysis detected the majority of the species present. This rarefaction analysis quantifies the number of taxa observed in subsampled data (Fig. 5). If the data has sampled all of the microbial groups present, then the curve of observed taxa vs. size of subsample will plateau, indicating that more data does not provide more information. For both the interior and surface samples, these curves approach a plateau, indicating that we sequenced enough to sample the majority of the taxa in and on concrete. This data was then used to calculate the Chao1 richness estimator, which uses the number of observed taxa in subsampled data to extrapolate the total number of taxa in a sample (Chao, 1984; Colwell and Coddington, 1994). Based on this estimator, we predict that approximately 1300 taxa are present in both samples. Since we observed approximately 1100 taxa in these samples, additional sequencing effort might identify additional rare taxa, but would probably not change the overall community composition described here.

The objectives of this phase of the research were to confirm that viable bacteria are present in concrete, develop a method for extraction of DNA from hardened concrete, and characterize bacterial communities in and on concrete. Here, we show that viable bacteria are present in concrete up to a year after pouring and curing, as demonstrated by successful cultivation and isolation of 15 different bacterial strains. We further demonstrate that DNA can be extracted from concrete and is pure enough to be amplified and sequenced, and that the microbial community in and on these concrete cylinders is dominated by two bacterial phyla, the Actinobacteria and Proteobacteria. The DNA extraction method can be used to characterize microbial communities in different types of concrete, in concrete exposed to different environmental conditions, or in corroding concrete.

The DNA sequence analysis also demonstrated that the interior of a concrete structure and its surface are inhabited by different microbes. This suggests that the bacteria in and on concrete are not just remnants.
of cells that were on the surfaces of the components (or in the water) at the time of mixing, but may instead reflect the different kinds of bacteria that survive in slightly different environmental conditions.

### 3.2 PHASE II: ASR BIOINDICATORS

In phase 2 of this research, we prepared 15 small test cylinders using concrete materials from the Delaware Department of Transportation (DelDOT) known to be highly ASR-reactive, and a parallel series of test cylinders that incorporated DelDOT’s standard mitigation against ASR. All cylinders were placed outside on a roof to weather, and one cylinder from each series was removed from the roof every 6-8 weeks and archived (Fig. 6). Three subsamples (5 g each) were collected from each cylinder and DNA was extracted from these. The 16S rRNA genes in these samples were then amplified and sequenced at the University of Delaware Sequencing and Genotyping Center, and the sequence data was analyzed using Qiime2 software. A total of 30 cylinders (15 from each series) was analyzed.

#### 3.2.1 Overview of sequencing results

A total of 7.9 million high-quality sequencing reads were obtained from 90 concrete samples (30 cylinders x 3 replicate subsamples of each), as well as the constituent materials and sterile glass beads. We removed any taxa observed in the glass beads, which we attribute to laboratory contamination: this is a conservative approach to the analysis, but because the goal was to identify taxa unique to ASR-reactive concrete, it is reasonable. On average, each subsample had 88,000 reads, representing nearly 60,000 individual taxa (Table 4). As in the Phase I study, Proteobacteria are abundant in the bacterial assemblages in concrete; in contrast to the Phase I data, Firmicutes were also abundant, while relatively few Actinobacteria were observed. When data from all ASR-reactive samples were pooled and compared to the pooled ASR-mitigated samples, it became clear that on the whole, the bacteria in these two kinds of concrete are quite similar: any gray lines in Fig. 7 indicate bacteria found in both sample types. Because the two concrete batches were made from the same materials, with the only difference being slag in the mitigated batch, we expected significant similarity, at least in the earlier samples. Some obvious differences between the sample types include some *Bacillus* and *Corynebacterium* species that seem to be unique to ASR-reactive samples (green lines in Fig. 7), and *Citrobacter* and *Cloacibacterium* species that may be unique to the mitigated samples (blue lines in Fig. 7).
<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Reactive/Mitigated</th>
<th>Sample date</th>
<th>Average temperature</th>
<th>Precipitation</th>
<th>Replicate 1</th>
<th>Replicate 2</th>
<th>Replicate 3</th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Reads</td>
<td>ASVs</td>
<td>Reads</td>
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<td>75445</td>
<td>59993</td>
<td>84161</td>
</tr>
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<td>63397</td>
<td>88774</td>
</tr>
<tr>
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<td>Mitigated</td>
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<td>56991</td>
<td>115578</td>
</tr>
<tr>
<td>5</td>
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<td>77250</td>
<td>56110</td>
<td>95566</td>
</tr>
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<td>6</td>
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<td>9/4/2013</td>
<td>74°F (53°-90°)</td>
<td>4.46°</td>
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<td>85532</td>
</tr>
<tr>
<td>9</td>
<td>ASR reactive</td>
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<td>28°F (3°-66°)</td>
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<td>85871</td>
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</tr>
<tr>
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<td>7.38°</td>
<td>98207</td>
<td>70866</td>
<td>83223</td>
</tr>
<tr>
<td>17</td>
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<td>62271</td>
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<td>4.74°</td>
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<td>5.50°</td>
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<td>56900</td>
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<td>2.95°</td>
<td>82804</td>
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<td>25</td>
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<tr>
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<td>3.32°</td>
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<td>86630</td>
<td>54272</td>
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</tr>
</tbody>
</table>
3.2.2 Sources of bacteria in concrete

One obvious question, when considering bacteria in or on concrete, is where those bacteria come from. To address this, we extracted DNA from triplicate subsamples of each of the components used to mix the concrete: coarse aggregate, fine aggregate, cement, and slag. The 16S rRNA genes from these samples were amplified and sequenced as for the concrete samples.

The SourceTracker software package (Knights et al., 2011; Hewitt et al., 2013) was used to compare the bacteria identified in the different components of concrete to those identified in the initial samples of the time series (Fig. 8). In the ASR-reactive (“Unmitigated”) samples, most of the bacteria are from an “Unknown” source. Because we did not sample the tap water used to mix the concrete in 2013, we cannot confirm that these bacteria originally came from the water; however, this seems like the most probable source, since the initial t<sub>0</sub> samples were never placed outside. In the slag-containing “Mitigated” samples, which should be less ASR-reactive, some of the bacteria seem to come from the slag itself, but the largest fraction of bacteria appears to come from the large aggregate. Although the sand was the component with the highest DNA yield and most bacterial diversity, it appears to contribute very little to the concrete microbiome.

3.2.3 Changes in bacterial assemblages over time

At a very broad level, it is clear that the bacterial assemblages in concrete change over time: the alpha diversity, a metric that incorporates both number of observed taxa and number of individual observations of each taxon, varies as a function of time. The five most abundant bacterial phyla, Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes, and Cyanobacteria, are present at fairly consistent levels throughout the time series, with slight upward trajectories (Fig. 9, top row). The next five most abundant phyla appear to vary seasonally, and there are differences between the ASR-reactive (unmitigated) and ASR-mitigated samples (Fig. 9, bottom row).
FIGURE 7. COMPARISON OF BACTERIA FOUND IN ASR-REACTIVE AND ASR-MITIGATED CONCRETE. All of the bacteria identified in any of the 30 concrete cylinders were quantified. Any taxa present in both types of sample at similar levels are indicated in gray; organisms colored green are more abundant in the ASR-reactive samples, while organisms colored blue are more abundant in the mitigated samples. This figure was made using Metacoder (Foster et al., 2017).
3.2.4 Differences between ASR-reactive and ASR-mitigated concrete

An ideal bio-indicator bacterium would be present in only ASR-reactive or ASR-mitigated samples at the end of the time series, would clearly change over time, and would be abundant enough to be reliably detectable. No species matching all these criteria have yet been identified. However, there are clear differences in the composition of and seasonal variability within the microbial assemblages in the two sample types. We first examined the species identified in Fig. 7 as being different in the ASR-reactive or unreactive samples, and plotted their abundance over time in all samples (Fig. 10). In most cases, although there are differences between ASR-reactive and mitigated samples that explain the results observed in Fig. 7, the differences at the end of the time series are not large enough to make these species reliable bio-indicators.

![Figure 8. Sources of bacteria in concrete.](image)

**FIGURE 8. SOURCES OF BACTERIA IN CONCRETE.** The bacteria identified in the concrete were compared to bacteria identified in each of the components, and the proportion of bacteria originating in each component, or from “unknown” sources, was predicted using Source-Tracker.

![Figure 9. Changes in phylum abundance over time.](image)

**FIGURE 9. CHANGES IN PHYLUM ABUNDANCE OVER TIME.** Relative abundance of each phylum is plotted as a function of date (month and year of collection are indicated in the x-axis labels). Some phyla, such as Cyanobacteria, appear to vary seasonally, while others such as the Bacteroidetes stay constant over time. Each point represents a species belonging to the indicated phylum (e.g. Proteobacteria); lines indicate averages of the phylum as a whole.
However, other species did have different patterns of variability over time. For example, the *Aerococcus* species appear to be similar for the first few months of the time series, as expected, since the two series were made with identical materials (the ASR-mitigated batch replaced half of the cement with slag, but that was the only change). In ASR-mitigated concrete, *Aerococcus* spp. appear to increase in January, peak in April, and decrease as the weather warms (Fig. 11). In ASR-reactive concrete, the same species decrease in January, but begin to increase in March and slowly increase through October. The *Lactobacillus* and *Exiguobacterium* spp., similarly, appear to be present at different abundances and respond differently to weather (Fig. 10). The differences between ASR-reactive and ASR-mitigated samples at the end of the time series make species within these two genera attractive as possible bio-indicators of ASR.
More useful than either of these are the *Staphylococcus* spp. (Fig. 1). These are consistently observed throughout the time series, suggesting that they are abundant enough to be reliably detectable. The seasonal dynamics in the ASR-reactive and ASR-mitigated samples are different, but the *Staphylococci* appear to increase over time in the mitigated samples, while they stay at approximately the same level in the unmitigated samples. Other genera currently under consideration as bio-indicators are *Aeromicrobium*, *Chitinophaga*, *Sediminibacterium*, and *Xenococcus*, all of which have different abundances in the two sample types at the final time point.

![Graph showing changes over time in selected differentially abundant genera](image)

**FIGURE 11. CHANGES OVER TIME IN SELECTED DIFFERENTIALLY ABUNDANT GENERA.** Relative abundance of each genus is plotted as a function of date (month and year of collection are indicated in the x-axis labels). Each point represents an observation of a species within the indicated group (e.g. *Bradyrhizobium*), and lines indicate averages of the group as a whole. Genera were selected based on apparent differences at the final time point.

## 4 CONCLUSIONS

### 4.1 GENERAL CONCLUSIONS

Based on the results from our Phase I (proof-of-principle) and phase II (time series) experiments, we have demonstrated that viable bacteria are present in concrete, and that DNA can be extracted from concrete and used to interrogate the concrete “microbiome.” We have further shown that most of the bacteria originate from the solid materials used in the concrete mix, and that the bacterial assemblages in concrete change over time. In a 2-year time-series comparing the bacteria in ASR-reactive and mitigated
concrete, the differences between the two sample types are minimal in the early time points, but increase over time. We observe seasonable variations in the bacterial communities in both sample types, and those variations are not identical. These results suggest that the bacterial communities in concrete depend on the composition of the concrete and are dynamic, changing in response to environmental conditions. Consequently, development of bacterial bio-indicators for specific types of chemical damage in concrete is feasible.

4.2 CAVEATS

Although we are hopeful about developing bio-indicators for early detection of ASR-induced damage, this research is still in an early stage. Some technical concerns include the possible presence of “relic DNA”, variability between samples, regional variations in materials used, heterogeneity within samples, and the extremely low biomass present in concrete. These will need to be resolved before bio-indicators can be used as a practical method for early detection of ASR or any other chemical degradation of concrete.

Concrete production is a very local industry: both coarse and fine aggregates, as well as the water used in the mix, are obtained from local sources. Since the majority of the bacteria identified in our samples came from the components used in the concrete mix (Fig. 8), it is possible that the bacterial communities in concrete produced in different regions, with different starting materials, could be quite different. Since concrete heats up as it cures, and since cured concrete is alkaline and dry, it is possible that these similarities result in similar bacterial communities, even if the bacteria in the components might be quite different. To determine whether there are regional differences in bacterial communities in concrete, or whether the similarity in concrete chemical and material properties results in a general “concrete microbiome,” bacterial communities from concrete from different regions would need to be characterized. If there are significant regional differences in concrete “microbiomes”, then unique bio-indicators would need to be identified in every region.

Potentially of even greater concern than variation between regional samples is the heterogeneity we observe within samples. In some cases, subsamples of the same concrete cylinder are as different from each other as they are from other cylinders. Because concrete is solid, and water flow through the matrix only occurs after the concrete has cracked, there is no mixing within the sample, and the intra-sample variability is very high. This has also been a consistent problem with analysis of microbial communities in soil samples (Lombard et al., 2011). As a result of this great heterogeneity, distinguishing between the species that change with respect to age or environmental variables and those that change with respect to location within a sample can be difficult. In soil systems, this problem can be solved by large enough sample sizes and by combining replicate samples, rather than analyzing them separately (Lombard et al., 2011). We anticipate that a similar approach will work here. The 5-g samples we collected have enough DNA to analyze, but it is very close to the limit of detection. Larger samples (10g or greater) might yield improved results, and data from replicate samples can be pooled to yield a more representative picture of the microbes found in each sample.

Another concern in this system is distinguishing between DNA from live cells, which can respond to changing environmental conditions, and DNA from dead cells. DNA is fairly stable even outside of the cell, and “relic DNA” can cause problems in this type of DNA-dependent analysis (Carini et al., 2016). In some cases, extracellular DNA can be degraded prior to extraction of DNA from cells, to prevent analysis of the relic DNA. However, because the crushing step in our protocol may break some cells open and release...
DNA, pre-treatment of extracellular DNA seems impractical. Instead, we are developing a method that uses confocal microscopy to visualize bacterial cells in concrete. We have successfully visualized *E. coli* on the surface of a small concrete chip (Fig. 12). If visualization of bacteria in and on concrete can be done using fluorescence microscopy, live/dead assays or activity assays that utilize dyes whose fluorescence properties change in response to biological activity (such as changes in pH or redox potential) can be used on these samples. This would allow us to determine how many of the cells in concrete are intact and active relative to those that have damaged membranes or are no longer active.

### 4.3 Proposed Bioindicators and Future Work

Our goal was to identify the bacteria present in ASR-reactive or mitigated concrete as the ASR damage developed and concrete weathered, then identify specific types of bacteria present at early stages of ASR damage. An ideal bioindicator bacterium would be present in only ASR-reactive or ASR-mitigated samples at the end of the time series, would clearly change over time, and would be abundant enough in all samples to be reliably detectable. In this data set, we have identified five candidate bio-indicators: *Staphylococcus, Aeromicrobium, Chitinophaga, Sediminibacterium*, and *Xenococcus*.

Additional testing will be required to determine the suitability of these organisms as bio-indicators in the field. These tests will include quantitative PCR (qPCR) against the 16S rRNA genes from these species in the existing DNA samples, to (a) confirm the results from the amplicon sequencing, and (b) more quantitatively determine the abundance of these species, since amplicon sequencing can be used for relative abundance but is not a reliable indicator of absolute abundance. The same qPCR procedure can then be used to quantify abundance of these species in ASR-damaged and undamaged samples obtained from local departments of transportation. Some samples have already been obtained from West Virginia and Delaware DOTs.

If the concerns outlined above can be overcome, it may also be possible to identify bioindicators for other types of damage. Additional work should also be done to determine whether different bioindicators would be necessary for reinforced concrete or for concrete with additives such as polymers that could potentially provide organic carbon to microbes.
REFERENCES


BMC Biol. **12**: 69.

testing methods and mitigation alternatives Austin, TX.


APPENDIX 1: INVITED SEMINARS PRESENTING THE FUNDED WORK.


5. **J.A. Maresca.** *Hard Microbiology: Bacteria in concrete.* Towson University, Department of Physics, Astronomy, and Geosciences, Towson, MD. April 8, 2016.


APPENDIX 2: CONFERENCE PRESENTATIONS OF THE FUNDED WORK


2. **J.A. Maresca***, J.L. Keffer. *Microbial communities in concrete and their potential application in ASR-damaged concrete*. Mid-Atlantic Transportation Sustainability Center – Region 3 University Transportation Center Annual Meeting, August 2016. (Poster presentation and invited talk.)

3. A.K. Treglia*, J.L. Keffer, **J.A. Maresca**. Using Microbial Populations in Concrete as Bio-Indicators of Alkali-Silica Reaction. American Society of Microbiology General Meeting/Microbe, June 2016. (Poster presentation)


5. K. Zhang, **J.A. Maresca**. Microbial communities in concrete and their potential application in ASR-damaged concrete. Mid-Atlantic Transportation Sustainability Center – Region 3 University Transportation Center Annual Meeting, August 2015. (Poster presentation.)


APPENDIX 3. PUBLICATION: MARESCA ET AL., MATERIALS AND STRUCTURES 2017
Analysis of bacterial communities in and on concrete

Julia A. Maresca · Paul Moser · Thomas Schumacher

Received: 23 February 2016 / Accepted: 6 July 2016 / Published online: 10 August 2016
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Abstract Bacteria are known to catalyze degradation of concrete, and have more recently been used to repair micro-cracks in or form protective biofilms on cement mortar. However, the microbial communities in and on concrete under ordinary weathering conditions have not been characterized, in part because of difficulty in extracting DNA from inside concrete specimens. Here, we report a method for extraction of nucleic acids directly from hardened concrete. Using this method and classical cultivation methods, we demonstrate that most bacteria in or on concrete belong to two taxonomic groups, that the bacterial diversity is similar on the concrete surface and in the interior, and that many bacteria in and on concrete are related to microbes found in other dry, saline, or alkaline environments. This method lays the foundation for the creation of bioindicators for concrete and may open new avenues for the fields of non-destructive evaluation and assessment of concrete structures.

Keywords Bacteria · Concrete · Bioindicator · Non-destructive evaluation

1 Introduction and motivation

1.1 Motivation

Worldwide, the concrete civil infrastructure network is aging and deteriorating, and agencies need inexpensive, effective and objective inspection techniques to make informed decisions with respect to maintenance and repair. In particular, early detection of damage is crucial, as deterioration processes tend to accelerate with time, along with the costs for rehabilitating a structure. Currently, condition assessments are mostly performed by using visual inspection methods, which can be subjective [1]. Non-destructive evaluation techniques are becoming more popular, but can be expensive due to the specialized equipment and expertise needed. Structural health monitoring allows for long-term collection of data using sensor networks attached to a structure. The challenge with this approach lies in the extensive analysis required to interpret the large amounts of continuously-produced data. Hence, there is a need for an inspection technique that is non-invasive, inexpensive, easy to deploy, and can detect conditions that may lead to deterioration early on.
Bioindicators are commonly used in water quality analysis [2] and food safety assessments [3] to identify contamination and/or assess risk, and we believe there is great potential for their application to non-destructive evaluation of concrete. The presence or activity of particular species of bacteria may indicate conditions that lead to deterioration mechanisms such as alkali-silica reaction (ASR), carbonation, or corrosion of the embedded reinforcing steel. To assess the potential for use of bioindicators in non-destructive evaluation, we must first confirm that viable bacteria are present in concrete, then develop a method for identifying these bacteria. The first steps toward identification of bioindicators for concrete are thus cultivation of representative bacteria from concrete, the development of a method for DNA extraction from concrete, and subsequent identification of microbial communities that naturally occur in and on concrete.

1.2 Background

In recent years, the effects of microbes on concrete degradation [4–6] and repair [7–9] have been characterized extensively. Microbial communities that degrade concrete have been characterized in detail in concrete sewers, where sulfur metabolism contributes to the acid-catalyzed dissolution of the structure [10, 11], and on submerged structures, where similar mechanisms operate [12, 13]. Next-generation sequence analysis has recently been applied to characterize surficial biofilms on these structures, and a variety of sulfur-metabolizing, acid-tolerant microbes were identified in these biofilms [14–17].

Additionally, bacterial precipitation of calcium carbonate (CaCO₃) has been tested as a mechanism for repairing small cracks in concrete, using urea ((NH₂)₂CO) or calcium lactate as substrates [8, 18], and encasing the bacteria or bacterial spores in silica gel or clay [8, 19]. Other work using microbial biofilms to prevent transport of ions into and out of cement mortar has shown that these surface-associated cells can increase concrete service-life [20–22]. Thus, bacteria are already known to play roles in both degradation and protection of concrete.

However, little to nothing is known about the bacteria that live in and on ordinary concrete: the population dynamics, the heterogeneity within and between different kinds of concrete, how the microbes survive the stresses imposed by the salty, alkaline environment, or even what kinds of microbes they are. In order to examine the composition of the communities of bacteria that develop in and on concrete, a cultivation-independent method for community analysis is necessary. To further characterize how the members of this community have adapted to the extreme conditions present in concrete, it is necessary to cultivate members of this community in the laboratory.

Here, we describe the isolation of 15 bacterial strains for laboratory investigation of the physiology of survival in concrete. We additionally demonstrate efficient extraction of DNA directly from concrete, and use high-throughput sequencing and analysis of the 16S rRNA gene to describe the composition of the microbial community in concrete exposed outside for ~12 months. In the future, this cultivation-independent analytical method can potentially be used to monitor changes over time, or even as a proxy for diagnosis of potential degradation risks.

2 Objectives

The objectives of this study were to confirm the presence of viable bacteria in concrete, develop a method for the extraction of DNA from hardened concrete, and characterize bacterial communities in and on concrete.

3 Materials and methods

3.1 Preparation and weathering of concrete

To assess whether ordinary pavement concrete could be colonized by microbes, concrete cylinders (152 × 305 mm) were cast in December 2011 using a Delaware Department of Transportation (DelDOT) Class B mix design (Table 1), which is typically used for concrete pavements. Crushed coarse aggregates had a maximum size of 19 mm.

After casting, the cylinders were cured in their plastic forms for 2 weeks, then uncapped and kept in a storage room at room temperature. In February 2012, two cylinders were placed on top of a University building in Newark, Delaware (USA) (Fig. 1a), exposed to weather and minimal human interaction (Fig. 1b). After 12 months of exposure, samples for
cultivation and DNA analysis were collected. Prior to sampling, cylinders were moved from the rooftop to a pre-sterilized sampling area. All tools were sterilized with 95% ethanol. The top surface of one cylinder was struck using a 3-in. masonry chisel and hammer until surface chips or larger chunks spalled off (Fig. 1c, d).

3.2 Scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM–EDS)

SEM–EDS was used to quantify the elemental composition of the concrete. The concrete cylinder was sliced into \( \approx 8 \) mm-thick horizontal sections, then sections from the middle of the cylinder were further cut into pieces approximately \( 1 \times 1 \) cm. The samples were attached to aluminum stubs and coated with

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Design compressive strength, fc'</td>
<td>20 MPa (2900 psi)</td>
</tr>
<tr>
<td>Design cement content (minimum)</td>
<td>334 kg/m(^3) (560 lb/yd(^3))</td>
</tr>
<tr>
<td>Design water-to-cement ratio, w/c</td>
<td>0.45</td>
</tr>
<tr>
<td>Required air content</td>
<td>4–7 %</td>
</tr>
<tr>
<td>Required slump</td>
<td>50–100 mm (2–4 in.)</td>
</tr>
<tr>
<td>Required admixtures (ASTM 2013)</td>
<td>A, D, E, F, G</td>
</tr>
</tbody>
</table>

Table 1 Specified requirements for DelDOT Class B concrete mix

Fig. 1 Exposure of cylinders to weather and subsequent sampling for cultivation, DNA analysis, and SEM–EDS. a. Concrete cylinders were placed on the roof of a university building. b. Cylinders were exposed to normal weather conditions, including wind and precipitation, for 1 year. c. The cylinder was broken for sampling from the inside for both cultivation and DNA analysis. d. Slices were also taken for SEM–EDS analysis
3 nm of carbon in a Leica EM ACE600 and kept under vacuum overnight prior to SEM analysis. Three independent samples were analyzed, and for each, 15–20 2 x 2 mm fields of view were imaged and analyzed. Elemental analysis was performed with an Oxford Instruments INCAx-act detector attached to a Hitachi S-4700 field-emission scanning electron microscope (FE-SEM).

3.3 Bacterial growth conditions and isolation and identification of bacterial cultures

Individual chips from the top and side surfaces or internal areas of the concrete cylinders were collected in sterile 50 mL centrifuge tubes or WhirlPak bags. For cultivation, TE (10 mM Tris, 1 mM EDTA) was added to the concrete pieces in centrifuge tubes. The samples were vortexed for 10 s and 25 μL of the buffer was spread on solid CM-A medium, a minimal, slightly alkaline medium developed for this study. CM-A contains (per liter), 1 g NaHCO₃, 1.44 g ammonium acetate, 0.12 g MgSO₄·7H₂O, 0.14 g KH₂PO₄, 0.17 g K₂HPO₄, 1 mL each Wolfe’s vitamin and trace minerals solutions [23], and 11 mL phosphate buffer (62.7 g KH₂PO₄ L⁻¹, 1.27 g K₂HPO₄·L⁻¹, pH 8.5). The pH of the medium was adjusted to 8.0. Additional samples were gently sonicated to remove cells from concrete pieces, and buffer was transferred to the media in the same fashion. The plates were incubated at room temperature for 2 weeks. Individual colonies were restreaked onto fresh CM-A media until axenic. Isolation was verified by microscopy and 16S rRNA sequencing. Once isolated, strains were archived at −80 °C in 10 % glycerol.

Colonies were scraped from solid media into TE and collected by centrifugation. DNA was extracted by bead-beating in extraction buffer (200 mM NaCl, 200 mM Tris, 20 mM EDTA, pH 8.0), followed by chloroform extraction and ethanol precipitation. No genomic DNA was obtained using this method.

3.4 DNA extraction methods attempted

DNA extraction from concrete was initially attempted using the MoBio PowerSoil and PowerMax DNA extraction kits (MoBio, Carlsbad, CA, catalog nos. 12888 and 12988, respectively), but no genomic DNA was obtained. A method used for extraction of DNA from endolithic communities was also attempted [26]. Briefly, pulverized concrete (1–2 g) was suspended in 5 mL buffer composed of 200 mM NaCl, 200 mM Tris, and 20 mM EDTA (pH ~ 8.0). Zirconium beads (0.1 mM), 2 mL 20 % SDS, and 5 mL chloroform were added and cells were vortexed at maximum speed for 3 min. Lysate was centrifuged for ~5 min and the aqueous phase was transferred to a fresh centrifuge tube, re-extracted with an equal volume of chloroform, then precipitated with 1 volume cold isopropanol and 0.1 volume 3 M ammonium acetate. The DNA pellet was then washed with 70 % ethanol, air-dried, and resuspended in TE (10 mM Tris, 1 mM EDTA, pH 8.0). No genomic DNA was obtained using this method.

3.4.2 DNA extraction protocol

Each 5-g sample was washed with 20 mL of 0.5 M EDTA, followed by vortexing and centrifugation, to remove divalent cations from the concrete powder [27]. The powder was then suspended in 15 mL of lysis buffer (40 mM EDTA, 50 mM Tris pH 8.3, 0.73 M sucrose) with 15 mg of lysozyme per gram of concrete powder and incubated at 37 °C with gentle agitation for 30 min. Next, 100 μL of proteinase K (20 mg mL⁻¹) and 1.5 mL of 20 % sodium dodecyl sulfate (SDS) were added and the solution was incubated at 56 °C for 2.5–3 h with gentle agitation. Membranes, proteins and cell debris were removed by extraction with 20 mL chloroform. To remove additional non-nucleic acid material, 1 mL of 1.95 M sodium acetate was added to the aqueous phase of each sample after extraction and mixed vigorously for 30 s [27]. The sample was then centrifuged and the aqueous phase was transferred to a
fresh centrifuge tube and re-extracted with 0.8 volume of chloroform. DNA was precipitated with 1 volume of isopropanol and 0.1 volume 3 M sodium acetate. The DNA pellet was washed with 70 % ethanol, and genomic DNA was then re-suspended in 25 μL of autoclaved ddH2O and stored at −20 °C.

To confirm that biomass is not lost during the EDTA wash, DNA was extracted from the EDTA used to wash the concrete using the MoBio PowerWater DNA extraction kit (MoBio catalog no. 14900).

3.5 Sequence analysis

Genomic DNA was sent to Research and Testing Laboratories (Lubbock, TX) for 16S rRNA gene amplification and sequencing. Primers for amplification of the V3–V5 region were 515F and 806R [28] with Illumina-specific primers and barcodes. Sequencing was performed on an Illumina MiSeq; both amplification and sequencing were done by Research and Testing Laboratories (Lubbock, TX).

Paired raw reads were joined, quality-filtered and assigned to taxa using Qiime pipelines [29, 30]. Operational taxonomic units (OTUs) were defined as clusters sharing 97 % sequence identity. Alpha and beta diversity parameters were calculated using standard Qiime pipelines [31].

4 Results and discussion

4.1 Concrete characteristics

An analysis using SEM/EDS was performed on small specimens of the weathered concrete to quantify the elemental composition (Fig. 2). Oxygen was the most abundant element, followed by Si, Al, Ca, K and Na. Small amounts (<1 % each) of Mg, Fe, and S were also detected in some areas of these samples. Because the samples were carbon-coated, carbon content could not be analyzed in this system. The Fe and S could potentially serve as electron donors or acceptors for microbial energy metabolism, and have been shown to do so in corroding concrete sewer pipes [10, 32].

4.2 Bacterial strains isolated

Fifteen bacterial strains were isolated from the surface and inside of the concrete samples. Greater diversity was initially observed on solid media spread with samples from surfaces than interior samples (Table 2). Individual colonies were transferred to fresh solid medium until the culture contained only one kind of organism, as assessed by microscopy and 16S rRNA amplification and sequencing.

Nearly all of the bacteria isolated from the concrete test samples are Actinobacteria, primarily Arthrobacter spp. (Table 3). One representative each of Alphaproteobacteria (a Paracoccus sp.) and Gammaproteobacteria (Pseudoalteromonas) were also isolated. Most of these strains are brightly colored due to the synthesis of carotenoid pigments (data not shown), and grow readily on both solid and liquid medium using acetate as the carbon and energy source. Relatives of several of these species were isolated from environments that are similar to concrete in salinity or alkalinity. For example, Kocuria species have been found in marine sediments [33] and salt evaporation works [34]. Dietzia natronolimnaea strains have been isolated from moderately saline alkaline lakes [35], and Nocardoides lentus has been isolated from alkaline soils [36].

4.3 Microbial community characterized by sequencing

4.3.1 Identification of a suitable DNA extraction method

Because ancient bone, like concrete, is dry, hard, and high in Ca²⁺, methods developed for extraction of
DNA from ancient bone were modified for application to concrete samples [27, 37]. Based on these methods, the sample is washed with EDTA prior to extraction to remove some of the divalent cations; later, additional salts are precipitated using a sodium acetate solution. Using this protocol, we consistently obtain high-molecular-weight genomic DNA from concrete (Fig. 3). This DNA is suitable for downstream processing by polymerase chain reaction (PCR) amplification and sequencing. No genomic DNA was observed after extracting DNA from the EDTA wash solution, confirming that this step does not result in appreciable loss of biomass from the samples.

4.3.2 General sequence quality

The 16S rRNA genes of the bacterial communities were amplified from DNA samples from the top and inside of the test cylinder (Fig. 1c). Sequences of this gene are standardly used to assess the taxonomic diversity of microbes in natural environments, as the 16S rRNA gene is both universally distributed and highly conserved [28, 38]. Amplicons were sequenced using Illumina MiSeq technology at the Research and Testing Laboratories in Lubbock, TX. 25,317 paired-end reads were obtained from the “top” sample, and 59,086 from the “inside” sample. The majority of these reads could be joined with their mate-pairs and passed subsequent quality filtering (Table 3).

4.3.3 Bacteria identified by sequencing

More than 250 bacterial genera were identified in the sequence data sets (Fig. 4; Supplementary Table 1). The bacterial community on the top surface was more diverse than that inside the concrete (Fig. 4), but the

### Table 2 Identification of bacteria isolated from concrete

<table>
<thead>
<tr>
<th>Strain designation</th>
<th>Phylum</th>
<th>Closest relative in GenBank</th>
<th>Colony color</th>
<th>Cell shape</th>
</tr>
</thead>
<tbody>
<tr>
<td>IN-02CB</td>
<td>Alphaproteobacteria</td>
<td><em>Paracoccus marinus</em> KKL-B9</td>
<td>Green–yellow</td>
<td></td>
</tr>
<tr>
<td>IN-02</td>
<td>Gammaproteobacteria</td>
<td><em>Pseudoalteromonas</em> sp. BSI20669</td>
<td>Orange</td>
<td>Short rod</td>
</tr>
<tr>
<td>IN-01</td>
<td>Actinobacteria</td>
<td><em>Arthrobacter</em> sp. 32c</td>
<td>White</td>
<td>Short rod</td>
</tr>
<tr>
<td>IN-02C</td>
<td>Actinobacteria</td>
<td><em>Kocuria marina</em></td>
<td>Green–yellow</td>
<td>Coccus</td>
</tr>
<tr>
<td>IN-03</td>
<td>Actinobacteria</td>
<td><em>Dietzia cecidiphylli</em> strain X10</td>
<td>Orange</td>
<td>Rod</td>
</tr>
<tr>
<td>IN-04</td>
<td>Actinobacteria</td>
<td><em>Rhodococcus cecidiphylli</em> strain EB37</td>
<td>Orange–yellow</td>
<td>Long rod</td>
</tr>
<tr>
<td>IN-06</td>
<td>Actinobacteria</td>
<td><em>Arthrobacter</em> sp. clone EK_Ca790</td>
<td>Pink</td>
<td>Rod</td>
</tr>
<tr>
<td>IN-06B</td>
<td>Actinobacteria</td>
<td><em>Kocuria marina</em></td>
<td>Yellow–green</td>
<td>Coccus (pairs)</td>
</tr>
<tr>
<td>IN-07</td>
<td>Actinobacteria</td>
<td><em>Arthrobacter agilis</em> strain DSM 20550</td>
<td>Pink</td>
<td>Coccus</td>
</tr>
<tr>
<td>IN-08</td>
<td>Actinobacteria</td>
<td><em>Nocardoides lentus</em> strain KSL-19</td>
<td>Burnt Orange</td>
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</tr>
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<td>IN-09</td>
<td>Actinobacteria</td>
<td><em>Dietzia natronolimnaea</em> strain NF047</td>
<td>Orange–red</td>
<td>Rod</td>
</tr>
<tr>
<td>CL-01</td>
<td>Actinobacteria</td>
<td><em>Dietzia natronolimnaea</em> strain NF047</td>
<td>Orange</td>
<td>Short rod</td>
</tr>
<tr>
<td>CL-02</td>
<td>Actinobacteria</td>
<td>Uncultured <em>Arthrobacter</em> sp. clone Acro223</td>
<td>Yellow–green</td>
<td>Coccus</td>
</tr>
<tr>
<td>CL-04</td>
<td>Actinobacteria</td>
<td><em>Arthrobacter</em> sp. 5139</td>
<td>White</td>
<td>Coccus (pairs)</td>
</tr>
<tr>
<td>CL-05</td>
<td>Actinobacteria</td>
<td><em>Rhodococcus erythropolis</em> strain zzx26</td>
<td></td>
<td>Coccus</td>
</tr>
</tbody>
</table>

All strains were identified by amplification of the 16S rRNA gene with primers 8F and 1492R and Sanger sequencing of the products. Strains designated “CL” were isolated from top or side surfaces of concrete; strains designated “IN” were isolated from internal samples.
most abundant groups in both were Actinobacteria and Proteobacteria (Fig. 4). In the inside sample, the most abundant Actinobacterial genera were the Micrococccaceae, which includes the Arthrobacter spp. and Nocardioidaceae that we isolated. Spore-forming Firmicutes (Bacillus and Clostridium spp.) are \( \approx 2.5 \% \) of the sequences in the inside sample; in total, Gram-positive bacteria comprise more than 65 \% of the species identified in the inside sample (Fig. 4; Supplementary Table 1). The Alphaproteobacteria were the most abundant Proteobacteria, specifically Rhizobia and Caulobacteria (both common soil bacteria). Nearly 6 \% of sequences could not be assigned to specific taxa.

In contrast, the Actinobacteria only comprised \( \approx 33 \% \) of the total community on the top of the cylinder. The Alphaproteobacteria and Betaproteobacteria species were much greater fractions of this community, though many of the Alphaproteobacteria could not be classified further (Fig. 4). Sequences related to cyanobacteria and chloroplasts (plant-associated organelles, likely coming from pollen in these samples) were more abundant on the top surface than inside. Putatively spore-forming Firmicutes were only small fractions of both communities, but were more abundant in the inside sample. Deltaproteobacteria related to Bdellovibrio and Myxococcus, which are predatory species that consume other bacteria, were observed only in the top sample (Fig. 4).

### 4.3.4 Potential effects of environmental conditions on microbial community

Although concrete is a relatively low-diversity environment (in comparison, the average soil microbial community has between 20,000 and 50,000 species per gram of soil [39]), we adequately sampled the diversity present in this environment (Fig. 5). The composition and limited diversity of the microbial communities in and on concrete likely reflect the sources of the bacteria, the chemical composition of the concrete, and the effect(s) of environmental conditions. Because environmental inputs to concrete (via rain, deposition, or fecal contamination) and weather conditions may change on a daily basis, the community composition likely also reflects the length of time that the concrete has been exposed. Similar to microbial biofilms on rock or monument surfaces, bacteria in and on concrete must be able to tolerate the fundamental properties of concrete (high pH, high salinity, low moisture content) as well as environmental conditions, which may change rapidly [40].

From February 2012 to February 2013, these test cylinders were on a university rooftop, where they were exposed to temperatures from -13 to 38 °C and a total of 95.5 cm of precipitation (data for February 10, 2012 to February 10, 2013 obtained from www.wunderground.com/history). The diurnal and seasonal variations likely selected for organisms tolerant to a wide range of conditions or for organisms capable of forming dormant cell types, which can withstand extreme environmental conditions.

Actinobacteria were the most abundant organisms in the concrete-associated bacterial communities characterized in this study, and are similarly prevalent in aerobic surface-associated microbial communities on rock surfaces [40]. The microbial communities on monuments exposed to weather, such as a 13th-century wall painting in Austria [41], and endolithic and epilithic communities in and on Mayan limestone monuments [42], are also broadly similar to those on concrete. In these environments, Actinobacteria and Proteobacteria are highly represented [41, 43–45]. However, many of those communities are dominated by photosynthetic Cyanobacteria or algae, and often have more spore-forming Firmicutes as well. In
contrast, in both the surface and interior communities of concrete, Firmicutes and Cyanobacteria are minor components. Cyanobacteria and chloroplasts comprise only \*1.5 \% of the community on the surface, and \*0.6 \% in the interior; Firmicutes are \*2.5 \% of the organisms inside this sample, and less than 0.3 \% of the organisms on the surface. The organisms identified in our test cylinders are related to bacteria that are tolerant of if not adapted to alkaline, saline, or desert environments. For example, bacteria in the Geodermatophilaceae group, which are known to inhabit desert soils and the surfaces and interiors of rocks [46, 47], comprise 3.5 \% of the community on the concrete surface and \*1.2 \% inside (Fig. 4; Supplementary Table 1). Similarly, \*1 \% of the bacteria both inside and on our concrete sample were members of the Skermanella genus, representatives of which have been isolated from airborne dust [48], desert soils [49] and heavy-metal-enriched soils [50]. The presence of these organisms suggests that some of the bacteria in concrete may come from the aggregate or sand, as well as atmospheric deposition.

Previous analysis of microbially-induced concrete corrosion has focused on surface-colonizing microbes on the walls and ceilings of concrete sewers [10, 14, 51, 52]. We observe very little overlap between the organisms found in the concrete cylinder samples described here, which are primarily Actinobacteria, and the acidophiles and sulfate-reducing bacteria found in corroding concrete systems [10, 14, 51]. Although one study found that Actinobacteria and Alphaproteobacteria dominated the microbial communities on concrete sewer surfaces [51], the species distribution was quite different, as the Actinobacteria in those samples were primarily Mycobacterium spp. and the Alphaproteobacteria were mostly Acidiphilium spp., neither of which represents more than 0.01 \% of the community in cylinders characterized here (Supplementary Table 1). The large-scale differences between microbial communities in and on dry concrete (this study) and those on intermittently-submerged sewer surfaces suggest that environmental conditions play an important role in development of the surface-associated concrete microbiome.

Because our test samples were kept on a green roof, they may also have been exposed to avian fecal matter. However, the sequences from the top surface do not have any of the major indicator species identified in gull or goose [53, 54]. We conclude that the inputs contributing either novel microbes or nutrients to these concrete samples included precipitation and atmospheric deposition, but likely not fecal material from birds.

### 4.3.5 Comparison of bacteria identified by sequencing and cultivation

The microbial community in the interior of the concrete sample had much greater actinobacterial and proteobacterial diversity than we isolated. Over 60 genera of Actinobacteria were detected by amplicon sequencing (Supplementary Table 1). All of the actinobacterial isolates belong to genera identified in the sequence data set. However, the genera represented in the culture collection are not necessarily the most numerically abundant in the sequence data set (Table 2; Fig. 4; our laboratory has isolated representatives of taxa with asterisks). Although the Corynebacteria are six of our 13 actinobacterial
isolates, they represent less than 0.3% of the sequences from the “inside” sample, and an even smaller fraction of the “top” sample (Supplementary Table 1). However, Micrococcales are well-represented in both the sequence data sets and in our collection of isolates. Better understanding how these isolates survive and grow in concrete will therefore give us representative information about the bacteria in concrete. The prevalence of Actinobacteria rather than the spore-forming Firmicutes was unexpected, since spores could enable cells to survive the harsh conditions in concrete. However, Actinobacteria have similarly resistant membranes and some species form cysts or other dormant cell types [55–57]. The carotenoid pigments in Actinobacteria have also been shown to protect the cells from temperature stress and salt stress [58], which suggests a possible role for the pigments in the isolates described here.

Although the organisms that we isolated represent abundant groups within the concrete microbial community, they represent only a small fraction of the diversity present. This discrepancy is common when comparing cultivation-dependent and cultivation-independent analyses of the same environment [59, 60]. Additional cultivation efforts using different media formulations optimized for the kinds of bacteria observed in this environment would likely result in isolation of more species, including the numerically abundant Alphaproteobacteria.

4.3.6 Alpha diversity analysis

To determine whether our sequence coverage was enough to detect the majority of species present, we performed rarefaction analyses in which the number of OTUs observed in subsampled data were quantified (Fig. 5). Both curves in the rarefaction approach a plateau, indicating that our sequencing effort was sufficient to sample the majority of the OTUs in concrete. This data was then used to calculate the Chao1 richness estimator, which uses the number of observed OTUs in subsampled data to extrapolate the total number of taxa in a sample [61, 62]. We calculate that approximately 1300 OTUs are present in both samples, and observe just over 1100 in both. Additional sequencing effort could reveal additional rare taxa, but would not change the overall community composition described here.

5 Conclusions

The objectives of this study were to confirm the presence of viable bacteria in concrete, develop a method for extraction of DNA from hardened concrete, and characterize bacterial communities in and on concrete. Here, we show that viable bacteria are present in concrete up to a year after pouring and curing, as demonstrated by successful cultivation and isolation of 15 different bacterial strains. We further demonstrate that DNA can be extracted from concrete and is pure enough to be amplified and sequenced, and that the microbial community in and on concrete is dominated by two bacterial phyla, the Actinobacteria and Proteobacteria. The DNA extraction method can be used to characterize microbial communities in different types of concrete, in concrete exposed to different environmental conditions, or in corroding concrete.

Acknowledgments This work was supported by grant # 12A01559 from the University of Delaware Research Foundation to JAM and TS. Additional support was provided by the Mid-Atlantic Transportation Sustainability University Transportation Center (MATS UTC). MATS UTC is funded by grant # DTRT13-G-UTC33 from the US Department of Transportation and matching funds organized by the Delaware Center for Transportation. We thank Keira Zhang for assistance with concrete processing. Additionally, the authors gratefully acknowledge Dr. Deborah Powell at the University of Delaware BioImaging Center for assistance with the SEM/EDS analysis, Dr. Brewster Kingham at the University of Delaware Sequencing and Genotyping Center for sequencing of the 16S rRNA genes of the isolates and the Research and Testing Laboratories in Lubbock, TX, for amplicon sequencing of the 16S rRNA genes from DNA extracted directly from concrete. We thank David Dodd at the Delaware Department of Transportation for concrete materials and mix designs, Dr. Jennifer Biddle and Dr. Farshad Rajabipoor for helpful discussions, and Gary Wenczel, Michael Davidson, and Dr. Jessica Keffer for technical assistance.

References


