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Molecular characterization of bacterial communities associated with sediments in the Laurentian Great Lakes

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ABSTRACT

The Laurentian Great Lakes freshwater ecosystem comprises a series of interconnected lakes exhibiting wide spatial differences in hydrogeochemistry, productivity, and allochthonous inputs of nutrients and xenobiotics. Bacterial communities associated with offshore sediments in this ecosystem have been rarely studied. We evaluated bacterial communities associated with sediments from 10 locations in four of the five Great Lakes (Lakes Superior, Michigan, Huron, and Ontario) by generating16S rRNA pyrosequence libraries. Pyrosequencing analysis revealed the presence of 26 bacterial phyla and proteobacterial classes among Great Lakes sediment samples. Actinobacteria, Acidobacteria, Betaproteobacteria, and Gammaproteobacteria were the most abundant groups of bacteria. Redundancy analysis was used to examine the role of sediment properties, including depth and chemical composition, in shaping bacterial community structure. One sample from Lake Huron was distinctly different from all other samples. Phylogenetic analysis revealed that the sample contained greater abundances of groups of bacteria associated with polluted environments. This study constitutes the most extensive examination of bacteria associated with Laurentian Great Lakes sediments and sheds useful insight into the microbial ecology of the Great Lakes.

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Introduction

In aquatic systems, sediment bacteria are widely recognized as major components of benthic food webs (Kemp, 1990). Sediment bacteria play an important role in organic matter decomposition and nutrient cycling (Billen, 1982; Jones, 1982; Jorgensen, 1983). Additionally, sediment bacteria affect overall benthic metabolism by regulating pH and redox conditions through their consumption of hypolimnetic oxygen (Jones, 1982), nitrate, and sulphate (Brezonik et al., 1987; Kelly et al., 1988). In recent years, cultivation-independent surveys employing sequence analysis of 16S small subunit rRNA genes within freshwater sediments have allowed for the identification of multiple bacterial phylotypes that appear to be ubiquitous in freshwater sediments (Briée et al., 2007; DeBruyn et al., 2009; Zwart et al., 1998). Unfortunately, knowledge of the structure of bacterial communities in deep, freshwater sediments is still very limited.

In addition to their importance in benthic foodwebs and organic matter decomposition, studies have indicated that Great Lakes sediments can harbor bacteria that may serve as important pathogens in lake ecosystems. Perez-Fuentetaja et al. (2011) used quantitative polymerase chain reaction (qPCR) to quantify *Clostridium botulinum* type E spores in Lake Erie sediments and determined that, although the abundance of the bacterial spores was patchy, sediments may serve as sources of the bacteria that could initiate food web transfer of this pathogen. Similarly, Rinta-Kanto et al. (2009) used both qPCR and culturing techniques to determine the abundance of *Microcystis* spp. in Lake Erie sediment samples and suggested that potentially toxigenic strains persist spatially and temporally in the sediment and that these populations may even act as bloom sources in large lake systems.

Although studies have investigated the presence of several bacterial species in Great Lakes sediments (DeBruyn et al., 2009; Perez-Fuentetaja et al., 2011; Rinta-Kanto et al., 2009), presently, no studies have been conducted to characterize diversity of bacterial communities associated with sediments in deep, offshore regions of multiple Great Lakes. Consequently, there is a definite lack of knowledge regarding geographic distribution of different bacterial taxa within Great Lakes sediments. Information on the composition and diversity of deepwater sediment bacterial communities in the Great Lakes will allow for a better understanding of how communities are influenced by environmental factors and the role sediment bacteria play in the ecology of the Great Lakes ecosystem. To this end, the aim of this study was to use 16S ribosomal RNA (rRNA) gene barcoded pyrosequencing to characterize the bacterial

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communities associated with offshore Great Lakes sediment from 10 locations in four of the Great Lakes (lakes Superior, Huron, Michigan, and Ontario) and to relate the structure of these communities to sediment chemistry and water depth.

Materials and methods

Sediment sampling and nutrient analysis

A single sediment sample was collected from each of 10 sites at depths between 50 and 230 meters below the lake surface (Fig. 1). All samples were collected in August 2008 by taking Ponar grabs of surface sediments (sampling area 25.1 × 25.1 cm/8.2 liters). Immediately after benthic samples were brought to the surface, undisturbed sediment samples were collected from the top centimeter of the benthic sample with a sterile 50-ml tube, and either placed in sterile 80% ethanol and stored at −20 °C for metagenomic analysis or immediately stored at −20 °C for nutrient analysis. Sediment phosphate (P), potassium (K), calcium (Ca), magnesium (Mg), nitrate-nitrogen (NO_3), and ammonium-nitrogen (NH_4) concentrations (Table 1) were determined according to Brown 1998 by the Michigan State University Soil and Plant Nutrient Laboratory (Department of Crop and Soil Sciences, Michigan State University, East Lansing, MI, USA). Water column data (Table 1) were taken from the United States Environmental Protection Agency (USEPA) as compiled in the Great Lake Environmental Database (GLENDA).

DNA isolation and 16S rRNA gene amplification

Genomic bacterial community DNA was extracted from sediment samples using the PowerSoil™ DNA Isolation Kit (MO BIO Laboratories Inc., Carlsbad, CA) following the manufacturer's protocol. DNA was quantified with a Qubit fluorometer and the Quant-it dsDNA BR Assay kit (Invitrogen Corp., Austin, TX). The isolated bacterial DNA was then used as a template for PCR amplification and pyrosequencing. Amplification of the V3–V5 region of the 16S rRNA gene was accomplished using the Broad HMP protocol (http://www.hmpdacc.org/). Briefly, barcodes that allow sample multiplexing during pyrosequencing were incorporated between the 454 adaptor and the forward primers. PCR amplification was conducted in duplicate 50-μl reactions. PCR reactions contained 5.0 μl of 10X AccuPrime PCR Buffer II (Invitrogen, Carlsbad, CA), 2 μM of each primer (357 F: 5′-CCTACGGGAGGCAGCAG-3′ and 926R: 5′- CCGTCAATTCMTTTRAGT-3′), 0.5 μl Accuprime Taq Hifi (Invitrogen), and 5.0 μl of template DNA. PCR reaction conditions for pyrosequencing samples were as follows: initial 95.0 °C for 2 min, followed by 30 cycles of 95.0 °C for 20 s, 50.0 °C for 30 s, and 72.0 °C for 30 s, and a final extension of 72.0 °C for 5 min. Previous PCR amplification of the same sediment DNA samples analyzed in the current study using quantitative polymerase reaction (qPCR) showed that 30 PCR cycles resulted in sub-plateau phase amplicons for the vast majority of the sediment samples (data not shown). PCR products were purified by using AmPure Beads (Agencourt Bioscience, Beverly, MA) following the manufacturer's protocol and then mixed equally (10 ng of each PCR product) before conducting pyrosequencing. The purified PCR products of the V3–V5 region of 16S rRNA gene were sequenced using the Roche 454 GS Junior System (Roche, Nutley, NJ, USA). To ensure reproducibility of pyrosequencing, rRNA genes from one sample (HU-95B) were extracted, amplified with different barcoded primers, and sequenced in triplicate. All sequences have been deposited in the NCBI short read archive (accession: bioproject: PRJNA198517, accession numbers: SAMN02645163–SAMN02645174).

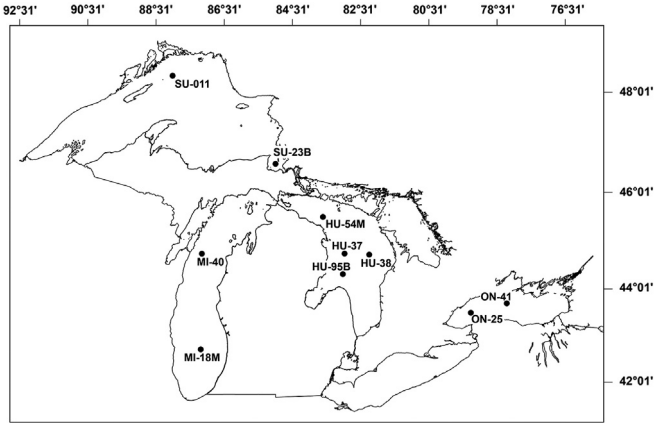

Fig. 1. Map of the Laurentian Great Lakes showing sampling sites for this study.

Sequence processing and library construction

Mothur software version 1.32.1 (Schloss et al., 2009) was used to denoise, trim, filter and align sequences, find chimeras (error sequence reads composed of at least two partial sequences from different real genes), and assign sequences to operational taxonomic units (OTUs) based on 97% sequence similarity. For taxonomic differentiation, 97% sequence similarity has been used to differentiate bacteria at the species level, 95% at the genus level, 90% at the family/class level, and 80% at the phylum level (Hugenholtz et al., 1998; Hughes et al., 2001; Sait et al., 2002; Stackebrandt and Goebel, 1994). Briefly, after extracting sequence, flow, and quality files from the raw Standard Flowgram Format (sff) file, the data were de-noised in Mothur using the method of Quince et al. (2009). Quality-filtered sequences (minimum length 200 bp, with no low quality or ambiguous bases, no more than 1 mismatch to the barcode and primer, and homopolymers of 8 bp as a maximum) were separated by primer and barcode, and then trimmed. Sequences were aligned to the SILVA-database reference alignment v 102 and sequences outside the most represented alignment space were removed. Chimeras were identified using the method of Edgar et al. (2011) as implemented in Mothur and removed. The remaining sequences were classified against the 16S rRNA Ribosomal Database Project (RDP) database, training set 9, using a k-nearest neighbor approach with a bootstrap cutoff of 80%. The average length of all bacterial sequences without the primers in the final data set was 283 bp. The resultant sequences were subjected to random subsampling to equalize the sequencing depth to 3845 reads per sample (1000 resamplings).

Data analysis

Species richness and diversity statistics including Good's Coverage, nonparametric richness Chao, and Shannon Index, were calculated for each sequence library using Mothur (97% sequence similarity). Additionally, Spearman correlation analysis was used to assess the association between sediment properties and bacterial community richness and diversity. The role of sediment properties and depth at the sampling sites in shaping bacterial community composition was evaluated with redundancy analysis (RDA), which is a type of constrained ordination. Prior to RDA, abundance values were standardized by expressing the abundance of each OTU as a percentage of total abundance for each sample. Additionally, to account for "blind sampling" and large numbers of absences in the data sets, abundance values were transformed using the Hellinger equation as suggested by Ramette (2007). RDA ordination graphs were used to visualize the relationship between bacterial communities and sediment properties. Analyses were conducted in R (R Development Core Team, 2010) using the VEGAN package (version 2.0-2) (Oksanen et al., 2013).

Results

A total of 3769 OTUs (97% sequence similarity) representing 26 bacterial phyla and proteobacterial classes were identified across all

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