Analysis of Microbial Diversity – A Novel Metagenomic Approach to Decipher Beach Microbiome

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Introduction:

The microbial ecology of recreational beach waters is one of the most underexplored ecosystems. It is frequently disrupted by anthropogenic activities, particularly in summer months. Shotgun metagenomics allows for accurate quantification of bacterial, fungal, parasitic, and viral genomes as well as identification of antibiotic resistance and virulence genes. Analysis of beach metagenomes will help not only to monitor but also to quantify microbial burden and their ecological relationship.

Antimicrobial resistance is a major public health challenge. Detection of genes encoding antimicrobial resistance in complex microbial ecosystems such as beach waters allows for both qualitative and quantitative analyses of community resistome and potential transfer to human commensal bacteria and pathogens. This can improve epidemiological surveillance and outbreak investigation of pathogens with emerging antibiotic resistance.

Virulence factors help bacteria to invade the host, cause diseases, and evade host defenses. The virulence genes in beach water may indicate the presence of pathogens, and therefore may likely adversely impact the health of children and immunocompromised individuals.

Whole genome shotgun metagenomics was used to detect and identify microbes, virulence and resistance genes, and to characterize the microbiomes of three Milwaukee area beaches. This novel approach not only deciphers biodiversity of microorganisms in recreational water, but likely will allow a greater understanding of their contributions on ecology, beach and human.

Methods:

A total of 18 individual pooled beach water samples were collected during the summer of 2016 at Milwaukee area beaches–Bradford (7), McKinley (5), and South Shore (6)–based on disparity between findings by Colilert, qPCR, and/or Nowcast predictive model.

Colilert-18 (IDEXX Lab.) was used to detect fecal coliforms. Lyophilized SmartBeads (BioGx, Inc.) were used for *E. coli* quantification via qPCR.

For shotgun metagenomics, extracted DNA was quantified using Qubit 4 Fluorometer (Thermo Scientific). Nextera XT library prep protocol was used to generate fragment libraries from DNA samples, followed by 150bp paired-end sequencing on Illumina HiSeq4000 (generated an average of 40M read pairs/sample).

CosmosID bioinformatics platform was used for multi-kingdom microbiome analyses and profiling of community resistome and virulome.

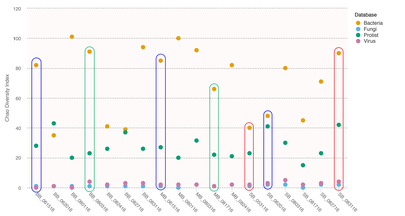
Table 1: Actual vs Expected Beach Water Quality Postings

Sample ID	Date of collection	Colilert (MPN/ 100 mL)	qPCR (CCE/ 100 mL)	Nowcast Model Prediction	Actual posting	Reason for posting	Expected posting	
BB061516	6/15/16	Open	Open	Close	Advisory	Rainfall 1"	Open	✖
BB062016	6/20/16	Open	Open	Open	Open	Colilert results from 3 days ago	Open	✔
BB080116	8/1/16	Open	Open	Open	Open	Colilert results from previous day	Open	✔
BB080316	8/3/16	Advisory	NP	Open	Open	Colilert results from previous day	Advisory	✖
BB082416	8/24/16	Open	Open	Open	Open	Colilert results from previous day	Open	✔
BB082716	8/27/16	NP: Weekend			Open	Colilert results from previous day	?	?
BB083116	8/31/16	Open	Open	Close	Advisory	Heavy rain/combined sewer overflow	Open	✖
MB061516	6/15/16	Advisory	Close	NP	Advisory	Rainfall 1"	Advisory	✔
MB080116	8/1/16	Open	NP	NP	Advisory	Colilert results from 3 days ago	Open	✖
MB080316	8/3/16	Open	NP	NP	Open	Colilert results from previous day	Open	✔
MB081716	8/17/16	Open	Open	NP	Advisory	Colilert results from previous day	Open	✖
MB082416	8/24/16	Open	Open	NP	Open	Colilert results from previous day	Open	✔
SB053116	5/31/16	Open	Close	NP	Open	Colilert results from previous day	Open	✔
SB062016	6/20/16	Close	Open	NP	Open	Colilert results from previous day	Close	✖
SB080316	8/3/16	Advisory	NP	NP	Open	Colilert results from previous day	Advisory	✖
SB081716	8/17/16	Advisory	Open	NP	Advisory	Colilert results from previous day	Advisory	✔
SB082716	8/27/16	NP: Weekend			Open	Colilert results from previous day	?	?
SB083116	8/31/16	Close	Close	NP	Advisory	Heavy rain/combined sewer overflow	Close	✖
Correct prediction	✔	Wrong prediction	✖	NP: Not performed	?			

Figure 1: Microbial Diversity in Milwaukee Beaches

Bradford (BB), McKinley (MB), South Shore (SB)
Species of bacteria (n=645), fungi (n=14), parasites (n=20), viruses (incl. bacteriophages) (n=21) identified. Chao diversity index given for bacteria, fungi, protists, and viruses.

Species Alpha Diversity



Beach posting as per Colilert (*E. coli* presence): Missed Advisory | Missed Closure | Expected Open

Results:

Figure 2: Bacterial diversity comparison based on dissimilarity between samples

Beta diversity principal coordinate analysis using Bray-Curtis method

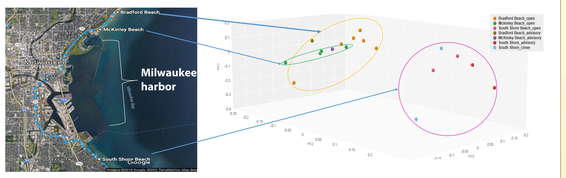


Figure 3: Fungi – Relative abundance shown as 100% stacked bar graph

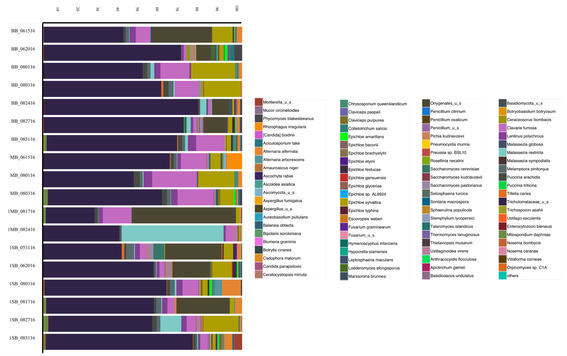
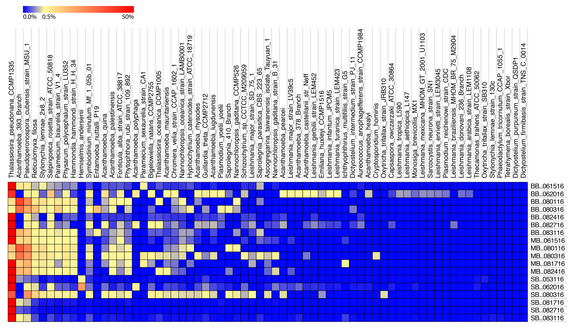


Figure 4: Protists – Relative abundance heat map


0.0%
0.5%
50%
Thalassiosira_pseudonana_CCMP1335
Aureococcus_anophagefferens_CCMP1709
Aureococcus_anophagefferens_CCMP1984
Aureococcus_anophagefferens_CCMP1850
Aureococcus_anophagefferens_CCMP1851
Aureococcus_anophagefferens_CCMP1848
Aureococcus_anophagefferens_CCMP1850
Aureococcus_anophagefferens_CCMP1850
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