Biodiversity of the Operational Aerosol Background

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Motivation

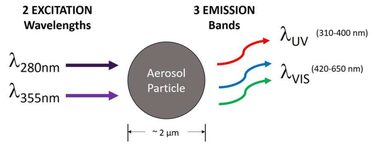
- Biological aerosol (bioaerosol) detection and identification systems must operate in challenging conditions. The natural aerosol background is complex and highly variable.
- It is important to characterize the aerosol background and understand how the environment impacts technology performance in the field. Specifically, for modern light induced fluorescence (LIF), polymerase chain reaction (PCR), and DNA sequencing systems.
- Background data helps technology developers design advanced algorithms and reduce false alarms. It also helps to develop operationally relevant test methods.

Aerosol Particle Monitoring

- Particle monitoring devices are housed inside a climate-controlled shelter stationed at Edgewood, MD, and Dugway , UT.

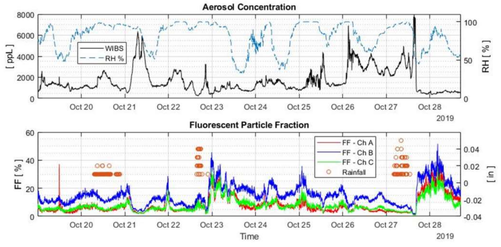
WIBS Fluorescence Particle Monitor

Multi-band LIF (3 channels, UV and VIS)



Particle Data Processing

- Data from the WIBS particle monitor is processed by counting the fluorescent and non-fluorescent particles and combined with meteorological data.



Physical Aerosol Particle Sampling

- Physical samples are collected onto dry filters using two devices: a SASS 3100 electret filter and a Dry Filter Unit (DFU). Filters are processed in the laboratory via PCR and DNA sequencing for metagenomics analysis.

SASS 3100 DFU

Nanopore DNA Sequencing

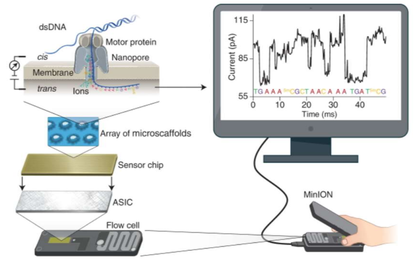
- Nanopore sequencing enables DNA/RNA sequencing in field environments.
- Workflows are quicker and easier to perform than traditional next-generation DNA sequencing methods.
- Long sequencing reads can be produced using this technology, which helps with genome assemblies and detection of genetically modified organisms.
- Untargeted DNA/RNA sequencing allows for the detection of any biological agent, including emerging threats.

Commercial DNA Sequencers

Oxford Nanopore Technologies

MinION (Handheld Flow Cell) **GridION** (High Throughput)

Nucleotides pass through the nanopore and produce a characteristic current change that is used to determine the corresponding nucleotide type at ~450 bases per second.



DNA Sequencing Results

- Filters were processed for aerosols collected in Mid-Atlantic and Desert environments, which show a very different organism composition.

Mid-Atlantic (Edgewood, MD)

Table 1. Top nine organisms at APG identified by nanopore sequencing

Organism	Genome Size	Reads Mapped	Unique Reads	% Unique Reads
Sphingomonas sp. PAMC26645 chromosome complete genome	4,283,956	535,432	239,367	44.7
Sphingomonas taxi strain ATCC 55669 complete genome	3,859,099	218,015	10,500	4.8
Pseudomonas fluorescens Pf0-1 complete genome	6,438,405	159,170	5,696	3.6
Pseudomonas syringae pv. syringae B728a complete sequence	6,093,698	119,502	4,706	3.9
Pseudomonas protegens Pf-5 complete sequence	7,074,893	103,690	234	0.2
Pseudomonas syringae pv. tomato str. DC3000 complete sequence	6,397,126	102,990	1,425	1.4
Sphingomonas wittichii RW1 complete sequence	5,382,261	85,636	836	1.0
Pantoea agglomerans strain C410P1 chromosome complete genome	4,182,028	82,323	535	0.6
Pantoea vagans C9-1 complete sequence	4,024,986	80,095	70	0.1

- Organisms identified in the Mid-Atlantic environment are commonly found in nature, and likely to be aerosolized. *Pseudomonas syringae* is a ubiquitous plant pathogen. Plants and foliage are abundant around the Edgewood, MD sample site.

Desert (Dugway , UT)

Table 2. Top nine organisms at DPG identified by nanopore sequencing

Organism	Genome Size	Reads Mapped	Unique Reads	% Unique Reads
Rubrobacter sp. SCSIO 52909 chromosome complete genome	4,378,772	9,723	6,037	62.1
Geodermatophilus obscurus DSM 43160 complete sequence	5,322,497	8,540	5,462	64.0
Botrytis cinerea B05.10 chromosome 4 complete sequence	2,468,882	5,375	4,011	74.6
Skermanella sp. W17 chromosome complete genome	5,869,433	4,986	3,066	61.5
Rubrobacter xylanophilus DSM 9941 complete genome	3,225,748	4,601	845	18.4
Nocardioides sp. JS614 complete sequence	4,985,871	3,169	504	15.9
Blastococcus saxobsidens DD2 complete genome	4,875,340	2,511	671	26.7
Microvirga ossetica strain V5/3m chromosome complete genome	5,843,140	2,458	1,136	46.2
Rubrobacter sp. SCSIO 52915 chromosome complete genome	4,156,492	2,277	1,148	50.4

- Two of the top organisms identified In the desert environment above (*Rubrobacter* and *Geodermatophilus obscurus*) are natural aerobic Gram-positive bacteria, which are found in, and survive in desert soil and rock surfaces. Another Gram-positive bacterium was also found among the top hits (*Blastococcus saxobsidens*), as well as a common plant fungus (*Botrytis cinerea*).

Lessons Learned and Future Directions

- Collection techniques and sample preparation methods were perfected and optimized for longer-tem background aerosol studies.
- This study provided a proof of concept and design which will be utilized for future larger-scale collections, including international environmental background studies with NATO collaborators.

Acknowledgements: Funding was provided by the Director, Combat Capabilities Development Command Chemical Biological Center under the authorities and provisions of Section 2363 of the FY 2018 NDAA to develop new technologies, engineer innovations, and introduce game-changing capabilities. Supplemental funding was provided by Joint Program Executive Office for Chemical, Biological, Radiological and Nuclear Defense (JPEO-CBRND).

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