

### **Potential Applications of Microbial Genomics in Nuclear Non-Proliferation (#18)** Isis Fukai PhD Student, University of Tennessee, Knoxville

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2. Away from reactor storage poo

# Introduction and Motivation

Existing methods for monitoring the nuclear fuel cycle (NFC) for potential proliferation concerns are largely limited to well controlled, near-source environments and simple sample matrices due to detector and analytical sensitivity. To complement, existing approaches, microbiome analysis could provide information about dynamic biological interactions and environmental conditions that control the transformation/distribution of nuclides released from fuel cycle activities.

Previous studies shed light on the diversity of microbial taxa and functional genes at various nuclear facilities and radionuclide contaminated sites. However, the microbial communities



reported at different sites vary widely, and characteristic taxa associated with specific stages of the nuclear fuel cycle have yet to be identified.

# **Mission Relevance**

This work presents a systematic review of microbial communities from nuclear facilities across the world to identify prokaryotic taxa commonly associated with each stage of the nuclear fuel cycle (Fig. 1). Findings are further evaluated via analysis of site-specific data from reactor facilities located at Oak Ridge National Laboratory and Savannah River National Laboratory. Identification of characteristic microbial taxa from specific stages of the NFC is intended to provide guidance for development of Wide Area Environmental Sampling programs, in-situ environmental biosensors, and geospatial analysis techniques for monitoring clandestine fuel cycle activities

# **Technical Approach**

In collaboration with partners at UC-Berkeley, previous studies examining prokaryotic communities from engineered and natural environments of 30 different nuclear facilities were reviewed (Table 1). Findings were compared and validated against site-specific



Figure 2. SRS study area and sample locations.

analysis of environmental samples collected from areas around R-reactor at the Savannah River Site (SRS) (Fig. 2), the High Flux Isotope Reactor (HFIR) at Oak Ridge National Laboratory (Fig. 3), and associated background locations (undeveloped, non-nuclear) at each site.



Figure 3. HFIR study area and sample locations.

# Results

### . List of the nuclear fuel cvcle facilities reviewed

		Facility		Radioactivity	
Fuel Cycle Stage	Country		Sample Media	(Bq/g or Bq/mL)	References
Mining/Milling,	Australia	Ranger	Soil	-	[1], [2]
Conversion	Bulgaria	Sliven	Soil	-	[3]
	Canada	Cluff Lake D/DJX pits	Surface water	-	[4]
		Key Lake/McArthur River	Sediment, surface water	-	[5]
	China	Shaoguan	Soil	-	[6]
		Yili/Yining	Soil	-	[7], [8]
	France	Bellezan/Brugeaud	Groundwater	0.4E-3 - 2.5E-3	[9]
	India	Bagjata	Ore, tailings	-	[10]
		Jaduguda	Ore, tailings	-	[10]
	Kazakhstan	Tortkuduk, Chu-Sarysu	Groundwater	-	[11]
	Sweden	Ranstad	Surface water	-	[12]
	USA	Edgemont	Soil	< 5	[13]
		Shiprock	Soil, surface water		[14], [15]
Fabrication	Russia	Siberia Chemical Combine	Groundwater	0.4 - 37	[16]
	USA	SRS A/300 M-Area	Soil	-	[17]
Reactor	Belgium	BR2 Reactor	Reactor tank/pool water	0.1 - 1.9	[18]
	France	Osiris	Reactor tank/pool water	3 - 3200	[19], [20], [21]
	Slovenia	TRIGA Mark II	Reactor tank/pool water		[22]
	Japan	Fukushima Daiichi	Soil (< 2 km from NPP)	95 - 519	[23]
	Ukraine	Chernobyl	Soil (< 2 km from NPP)	61 - 750	[24], [25], [26]
	USA	SRS R-Reactor	Soil (< 2 km from reactor)	-	[17]
Interim Spent	Brazil	Angra 1 <sup>1</sup>	pool water, biofilm	512	[27]
Fuel Storage	India	Madras Atomic Power Station <sup>1</sup>	pool water	-	[28], [29]
Pool	Spain	Cofrentes <sup>1</sup>	pool water, biofilm	1 - 500	[30], [31]
	Slovakia	JAVYS ISFS Facility <sup>2</sup>	pool water	1	[32], [33]
	UK	Sellafield Magnox Storage Pond <sup>2</sup>	open-air pool water	100 - 2000	[34]
		Sellafield Fuel Handling Plant <sup>2</sup>	pool water	1125	[35]
	USA	SRS L-Area Disassembly Basin <sup>2</sup>	pool biofilm, precipitate	-	[36]
Reprocessing	Russia	Mayak RT-1	Groundwater	0.1 - 3.3	[37]
	USA	Hanford S-SX Tank Farm	Vadose zone sediment	0.4 - 2.0E6	[38]

Pseudomonas, Bacillus, and Sphingomonas are the most highly reported genera across all stages of the NFC (Fig. 4). Geobacter, Acidovorax, Acinetobacter, and Arthrobacter are associated with the front-end facilities reviewed, whereas Methylobacterium, Staphylococcus, and Brevibacillus are reported at more reactor and back-end facilities.

Site-specific analysis shows higher relative abundances of methylotrophic Alphaproteobacteria\*, Bacillus, Sphingomonas, and Brevibacillus and/or Paenibacillus at HFIR and SRS reactor sites relative to background sites (Fig. 5). A higher percentage of *Staphylococcus* is also observed in SRS samples. In contrast, Bradyrhizobium is more abundant in background samples from each site.





Figure 6. Maps showing higher abundances of (A) an uncultured Methyloligellaceae genus and (B) Brevibacillus occurring around reactor effluent sources, following similar spatial trends as lanthanum and manganese.

Distributions of an uncultured methylotrophic genus (Methyloligellaceae family) and *Brevibacillus* effectively identify locations of reactor effluent sources, with higher abundances occurring closer to the reactor stack and cooling tower (Fig. 6A,B). Similar spatial trends are observed between the methylotrophic genus and lanthanum concentrations and between Brevibacillus and manganese, suggesting lanthanide-dependent methylotrophy and Mn transport systems in each respective genera may promote their fitness in reactor and back-end fuel cycle environments (Fig. 6 C,D; Fig. 7). 0.0

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Figure 5. Key taxa associated with reactor and back-end NFC facilities show higher relative abundances in (A) HFIR and (B) SRS samples compared to background samples.



Figure 7. Correlation between lanthanum and the methylotrophic bacterium in HFIR soil.

Characterization of representative microbial taxa associated with specific stages of the NFC provides import insights on the micro-to-macro-scale processes that govern outcomes of safeguards implementation, monitoring technologies, and contaminant mitigation strategies for the nuclear industry.

Results provide a framework for development of Wide Area Environmental Sampling programs, bio-indicators, and geospatial analysis techniques for monitoring clandestine fuel cycle activities.

In addition, the MTV Consortium has provided extensive support for:

- networking opportunities

Prokaryotic taxa commonly associated with each stage of the nuclear fuel cycle have been identified via review of microbial communities from 30 nuclear facilities in 18 different countries. Site-specific analyses provide further support of findings, including:

- cycle facilities

- metagenomics
- team Describe plans for ongoing work



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### **Expected Impact**

• Interdisciplinary dissertation research, training, workshop participation, and

 Collaboration with ORNL, SRNL on sample collection and laboratory analysis; UC-Berkeley (H. MacGregor, A. Arkin,) on data integration and modeling

## Conclusions

• Identification of methylotrophic genera, *Bacillus*, *Sphingomonas*, and Brevibacillus/Paenibacillusas as key taxa associated with reactor and back-end fuel

• Spatial distributions of methylotrophic genera and *Brevibacillus* can be used to effectively identify locations of reactor effluent sources

Lanthanide-dependent methylotrophy and Mn-transport systems may promote the fitness of methylotrophic genera and *Brevibacillus* in reactor environments

### **Next Steps**

• Evaluate genes and functional potential of microbiomes at each site via shotgun

Integrate data with machine-learning and computational models from UC-Berkeley

• Ongoing collaboration with Consortium members from ORNL, SRNL, and UC-Berkeley on model development, laboratory analysis, and technology transfer.

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