

DOE-ID NEPA CX DETERMINATION

Idaho National Laboratory

SECTION A. Project Title: Biological Persistence in the Environment

SECTION B. Project Description and Purpose:

The purpose of this work is to observe the persistence of microorganisms, like the yeast strain *Saccharomyces cerevisiae* NE095 (Risk Group 1, RG-1) in the environment. The test work will be performed in both lab and field settings. Lab settings will use soil samples collected from the site to develop microorganism sampling protocols. Future work will include validating the sampling protocols at Idaho National Laboratory (INL) desert locations at 43.866436, -112.706486. Samples will be collected by driving existing roads to as close as possible and then walking the remainder of the distance to the sample location. The lab work will be conducted at INL Research Center (IRC) IF-603, labs A-5, A-8, A-9, B-7, B-8 and B-9. In the lab, methods for applying and recovering cells from the collected soil will be developed. Additionally, protocols for establishing cell viability through plating methods will be established. Protocols for the identification of NE095 by PCR and qPCR methodologies will also be established. The techniques developed here form the basis of future sampling protocols to be used by first-responders to test for potential pathogens released in a bioweapon event. Tasks utilized for this work will include:

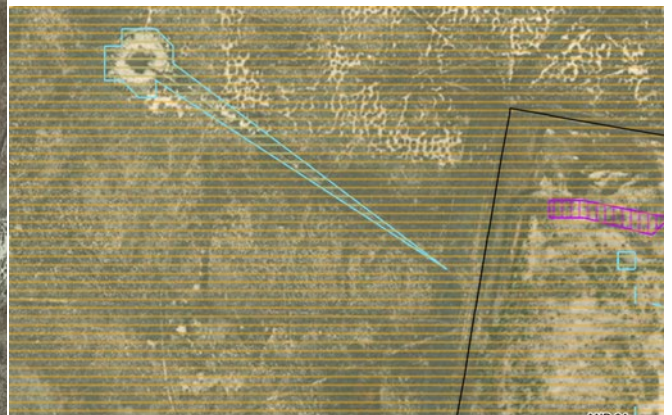
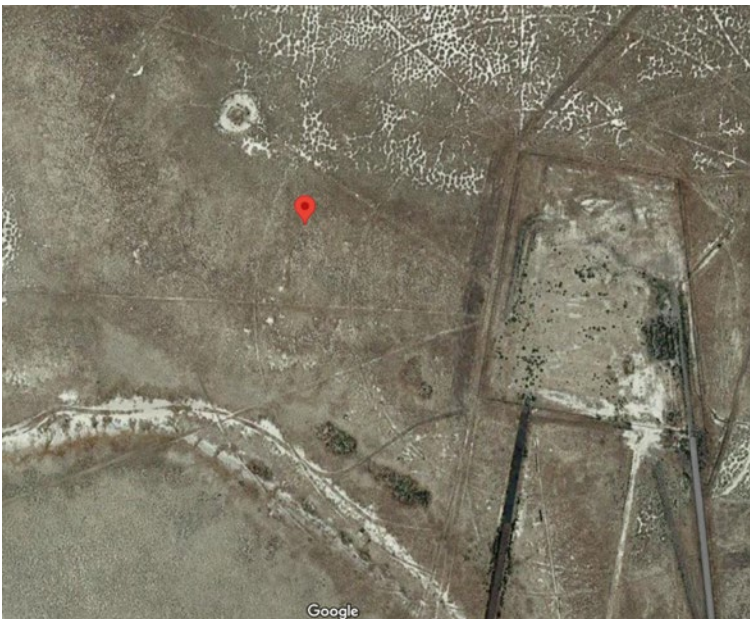
- Cultivation, including enrichment and isolation of RG-1 microorganisms
- Soil collection
- Applying and eluting microorganisms with a typical gravity flow column or alternative column set up
- Diluting and plating eluted microorganisms to test for viability
- DNA extraction and quantification for identifying viable microorganisms
- Microscopic observation and photography for microorganism characterization and counting
- Use of chemical laboratory techniques
- Processing of cell mass and DNA through cell culturing, cell isolation, DNA and isolation from cell lysis
- Soil samples will be collected and may be homogenized, sieved and/or sterilized before loading into sterile gravity columns for loading of cell culture

Experiments will be performed using good microbiological practices which are outlined in Appendix C of LWP-14621 "Laboratory Biological Experimentation Safety" and are summarized in Appendix A.

LI scope bounding conditions for activities:

- Will not include the cultivation of microorganisms from Risk Group 2 or higher
- Will not include radiological activities
- Will not include the use of vectors or genetic systems that induce the production of toxins or lead to pathogens or contain antibiotic markers

Expected waste includes spent cultivation media (liquid and agar plates), biological buffer used for column elution. The exact composition of the buffer used in the soil column studies has not been determined. It's likely to be a common biological buffer at a circumneutral pH. DNA extraction waste produced from extraction kits will be handled according to Waste Generator Services (WGS) disposal requirements. There will be spent cell culture media and media loaded onto soil. In both cases, when experiments are completed, these will be autoclaved and pH'd to between 4 and 12 and then poured down the drain. No equipment is anticipated to be purchased.



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SECTION C. Environmental Aspects or Potential Sources of Impact:

Air Emissions

Collecting soil samples may generate fugitive emissions.

Discharging to Surface-, Storm-, or Ground Water

N/A.

Disturbing Cultural or Biological Resources

Biological resources apply to activities that have the potential to interact, disturb or affect wildlife or their habitat. When ground disturbing activities are performed, even in previously disturbed soils, there is potential to impact cultural resources. Surveying will need to be conducted to follow Migratory Bird Act guidelines in refraining from impacting biological resources and to determine the amount of sagebrush that may be impacted.

Generating and Managing Waste

Expected waste includes spent cultivation media (liquid and agar plates), biological buffer used for column elution. The exact composition of the buffer used in the soil column studies has not been determined. It's likely to be a common biological buffer at a circumneutral pH. DNA extraction waste produced from extraction kits will be handled according to WGS disposal requirements and waste associated with PCR and qPCR will be very small quantities and will be disposed of as required by WGS. There will be spent cell culture media and media loaded onto soil. In both cases, when experiments are completed, these will be autoclaved and pH'd to between 4 and 12 and then poured down the drain.

Releasing Contaminants

Chemicals will be used and will be submitted to chemical inventory lists with associated Safety Data Sheets (SDSs) for approval prior to use. All chemicals will be managed in accordance with laboratory procedures. When dispositioning surplus chemicals, project personnel must contact the facility staff for disposition instructions. When chemicals are used during the project there is the potential for spills that could impact the environment (air, water, soil).

Using, Reusing, and Conserving Natural Resources

All materials will be reused and recycled where economically practicable. All applicable waste will be diverted from disposal in the landfill where conditions allow.

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SECTION D. Determine Recommended Level of Environmental Review, Identify Reference(s), and State Justification: Identify the applicable categorical exclusion from 10 Code of Federal Regulation (CFR) 1021, Appendix B, give the appropriate justification, and the approval date.

For Categorical Exclusions (CXs), the proposed action must not: (1) threaten a violation of applicable statutory, regulatory, or permit requirements for environmental, safety, and health, or similar requirements of Department of Energy (DOE) or Executive Orders; (2) require siting and construction or major expansion of waste storage, disposal, recovery, or treatment or facilities; (3) disturb hazardous substances, pollutants, contaminants, or Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA)-excluded petroleum and natural gas products that pre-exist in the environment such that there would be uncontrolled or unpermitted releases; (4) have the potential to cause significant impacts on environmentally sensitive resources (see 10 CFR 1021). In addition, no extraordinary circumstances related to the proposal exist that would affect the significance of the action. In addition, the action is not "connected" to other action actions (40 CFR 1508.25(a)(1) and is not related to other actions with individually insignificant but cumulatively significant impacts (40 CFR 1608.27(b)(7)).

References:

The R&D activities identified in this ECP is a CX, B3.6 "Small-scale research and development, laboratory operations, and pilot projects" and B3.1 "Site characterization and environmental monitoring."

Justification:

The proposed R&D activities are consistent with CX B3.6 "Siting, construction, modification, operation, and decommissioning of facilities for small-scale research and development projects; conventional laboratory operations (such as preparation of chemical standards and sample analysis); small-scale pilot projects (generally less than 2 years) frequently conducted to verify a concept before demonstration actions, provided that construction or modification would be within or contiguous to a previously disturbed area (where active utilities and currently used roads are readily accessible). Not included in this category are demonstration actions, meaning actions that are undertaken at a scale to show whether a technology would be viable on a larger scale and suitable for commercial deployment."

The soil sampling activities fit within the classes of actions listed in items CX B3.1, "Site characterization and environmental monitoring (including, but not limited to, siting, construction, modification, operation, and dismantlement and abandonment of characterization and monitoring devices, and siting, construction, and associated operation of a small-scale laboratory building or renovation of a room in an existing building for sample analysis). Such activities would not have the potential to cause significant impacts from ground disturbance. Covered activities include, but are not limited to, site characterization and environmental monitoring under CERCLA and RCRA. (This class of actions excludes activities in salt water and freshwater. See B3.16 of this appendix for salt water and freshwater activities.) Specific activities include, but are not limited to:

- (a) Geological, geophysical (such as gravity, magnetic, electrical, seismic, radar, and temperature gradient), geochemical, and engineering surveys and mapping, and the establishment of survey marks. Seismic techniques would not include large-scale reflection or refraction testing;
- (b) Installation and operation of field instruments (such as stream-gauging stations or flow-measuring devices, telemetry systems, geochemical monitoring tools, and geophysical exploration tools);
- (c) Drilling of wells for sampling or monitoring of groundwater or the vadose (unsaturated) zone, well logging, and installation of water-level recording devices in wells;
- (d) Aquifer and underground reservoir response testing;
- (e) Installation and operation of ambient air monitoring equipment;
- (f) Sampling and characterization of water, soil, rock, or contaminants (such as drilling using truck- or mobile-scale equipment, and modification, use, and plugging of boreholes);
- (g) Sampling and characterization of water effluents, air emissions, or solid waste streams;
- (h) Installation and operation of meteorological towers and associated activities (such as assessment of potential wind energy resources);
- (i) Sampling of flora or fauna; and
- (j) Archeological, historic, and cultural resource identification in compliance with 36 CFR part 800 and 43 CFR part 7."

Is the project funded by the American Recovery and Reinvestment Act of 2009 (Recovery Act) Yes No

Approved by Jason L. Anderson, DOE-ID NEPA Compliance Officer on: 11/15/2022