

OBSERVATORY FOR SUBSURFACE GEOMICROBIOLOGY
(A Working Observatory for Biogeochemical and Bioremediation Education and
Research into the Subsurface.)

EXECUTIVE SUMMARY

An Observatory for Subsurface Geomicrobiology (OSG) will be the only facility in the world where long-term, in situ geomicrobiology and biogeochemistry experiments explore the evolution, adaptation, and limits of microbial life, as we understand it, in the deep subsurface. The OSG will provide a here-to-fore previously unattainable resource for multi-disciplinary and multi-institutional investigations for the international earth and biological scientific communities. The primary goal of OSG is to provide an experimental and intellectual foundation for investigating the origin and bounds of life as well as to develop practical applications for the bioremediation, biotechnology and pharmaceutical industry. Integral to the scientific goals of the OSG will be a very active program to foster education and training for future generations of scientists and teachers from K-12 to visiting researchers, focusing on those groups that have remained underrepresented throughout the 20th century. To accomplish its goals the activities of the OSG must be closely linked and integrated with those of other earth science disciplines, including hydrology, geophysics, geology, geochemistry and rock mechanics and with other regional academic centers in order to take full advantage of shared technological infrastructure, intellectual prowess and educational outreach capabilities. The principal requirement for OSG is a large-scale underground excavation where drilling and coring can take place, preferably an abandoned, deep mine or a custom excavated facility down to a depth of at least 2.5 kilometers.

PART 1-RESEARCH THRUSTS AND EDUCATIONAL OPPORTUNITIES

The relatively recent discovery of deep subsurface microbial communities and what appears to be a subsurface biosphere has opened a new scientific frontier where earth sciences, chemistry, physics and biology merge to provide insights into how life on this planet and even extraterrestrial life, may have originated and evolved over billions of years (Fredrickson and Onstott, 1997). The geological isolation of these deep subsurface microbial communities offers the potential to answer questions on the origin of life and its diversity as well as constraining the possibilities for life beneath the surface of Mars and other planetary bodies. In addition to the role of microorganisms in shaping the life forms on earth, the importance of microorganisms in the dissolution and formation of minerals is only now becoming recognized as geomicrobiology comes to the fore. Advances in our understanding of the origins, diversity, distribution and function of microorganisms in deep, often extreme, subsurface environments will rapidly expand our knowledge of geomicrobiological and biogeochemical processes on Earth and beyond. The discovery of novel microorganisms from deep accessible subsurface habitats provides opportunities for discovering new pharmaceuticals, processes for biochemical and chiral-specific synthesis, environmental remediation and energy production. Finally, a fundamental knowledge of subsurface biogeochemical processes and elemental cycling

is critical for predicting the impacts of subsurface contamination and underground waste isolation and for development of subsurface remediation strategies, including the storage of radioactive waste and CO₂ sequestration. Indeed, this knowledge will be enabling for good environmental stewardship practices that will serve our posterity and us.

A major obstacle to understanding the subsurface biosphere has been our limited ability to access the deep terrestrial environment, acquire uncompromised samples and to place our knowledge of isolated microorganisms into the context of the geochemical and hydrogeological processes that control their existence, function and transport. In addition, opportunities to address biogeochemical and microbial transport in compromised but well-characterized deep fractured rock aquifers have been limited and have been restricted to geographically dispersed locations, markedly constraining the collaboration between disciplines critical to understanding complex interrelated phenomena.

The OSG will provide a unique opportunity to overcome accessibility and intellectual obstacles by offering 1) three dimensional access to a large-scale subsurface environment that has been highly characterized for over 125 years; 2) a multidisciplinary, collaborative environment that ensures cutting-edge measurements essential to design and interpretation of microbial studies (e.g., high sensitivity isotopic tracer detection, laser and infrared imaging of chemical and physical properties); and 3) an international, multi-institutional research and development and educational environment. These attributes, along with the ability to conduct long-term experiments, the ability to replicate those experiments in similar subsurface environments and the ready access to on site instrumentation for real-time detailed biological and chemical interrogations would establish OSG as the world leader in subsurface geomicrobiology research. A recent report on geobiology by Nealson and Ghiorse (2001) emphasized the need for establishing field laboratories for geomicrobiological research that are available for long-term studies; a need that OSG can fulfill.

RESEARCH THRUSTS

The proposed research thrusts of the OSG will focus on three major areas (and potential applications thereof); a) subsurface biological diversity, b) subsurface biogeochemical processes and c) subsurface microbial ecology.

A. Subsurface biological diversity

Subsurface biological diversity focuses on the discovery of new microorganisms, novel biological processes and microbial products with potential applications in pharmaceutical (e.g. antimicrobial agents), feedstock chemicals (e.g. chiral synthesis), bioremediation of industrial waste, industrial processing, nanotechnology, opportunities for comparative genomics/proteomics that will provide new insights into the mechanisms of prokaryotic and eukaryotic development and new technologies designed to detect and quantify these processes. Some of the hypotheses to be tested include those below.

A1. “The unique environments of the deep subsurface create microorganisms with unique antibiotic properties and other metabolic products that are important pharmaceuticals. (M. DeFlaun, Envirogen, Inc.; T. Hazen, Lawrence Berkeley National Laboratory)”

A natural adaptation to living in environments that are increasing recalcitrant is the incorporation and horizontal transfer of plasmids that code for enzymes capable of degrading more complex compounds, e.g. humics, that are normally recalcitrant. These plasmids produce the same enzymes that are responsible for many antibiotic activities. While an increasing plasmid load puts an obvious strain on bacteria in more dynamic near surface environments, it has the advantage of providing more carbon and energy sources in the deep subsurface. Indeed, we have also observed and increasing ability to use a broader range of carbon sources as we move deeper into the subsurface (Fredrickson et al; 1988; Jimenez, 1989). These phenomena were observed in ‘aseptic’ drilling studies down to 1700’ done by the DOE Subsurface Science Program in South Carolina. More recent bioprospecting for drug discovery has turned its sights to the unique ecosystems populated by extremophiles (Adams et al., 1995). The diversity of habitats and uniqueness of these ecosystems yields microorganisms that can function under conditions previously thought uninhabitable. Several companies have based their business on bioprospecting (Diversa, Teragen, Chromosome), and consider these extreme environments to be fertile hunting grounds, because the microbes that have evolved in these environments would have developed unique or yet to be discovered metabolic properties.

A2. “Microbial communities that inhabit the mining-impacted environments of the subsurface have properties that would make them uniquely suited to act as agents for ore processing or contaminant remediation. (M. DeFlaun, Envirogen, Inc.)”

Reduced, sulfidic ores when exposed to oxygenated, wet conditions produce a leachate that is typically acidic and contains high concentrations of toxic metals. Aerobic microorganisms derive energy from this environment, become adapted to its lethal conditions and even promote its development. In the presence of organic matter and suboxic conditions, the same leachate provides a source of energy for anaerobic microorganisms to flourish. Within old stopes an abandoned precious metal mine where these two processes are occurring, aerobic microorganisms may exist that can be adapted to large-scale industrial processing of metallic ores. Just such a strategy has proven quite profitable for Biox of South Africa. Metal resistant anaerobic microbial communities could also be present that can be utilized to remediate toxic metal contaminated groundwater or mine wastes.

A3. “High temperature, highly saline groundwater harbors microorganisms with unique metabolic properties that are useful in biotechnological applications (M. DeFlaun, Envirogen, Inc.)”

Microorganisms that are capable of surviving at high temperatures in highly saline aqueous solution and extracting energy from recalcitrant organic or inorganic compounds have the ability to maintain their enzymatic proteins under energy deficient conditions. This environment may promote the selection of microorganisms with proteins that are temperature and salinity tolerant. Such proteins have enormous potential for industrial processes.

A4. “The physiological and genetic diversity of subsurface microbial community members will be a function of habitat characteristics. The more diverse the available energy resources and electron acceptors in a given habitat, the higher will be the level of biological diversity (J.K. Fredrickson, Pacific Northwest National Laboratory).”

A4.1. “The highest degree of community complexity and microbial diversity will occur in the mine-altered, subsurface environments. The influx of air as a result of ventilation will promote development of complex microbial communities based on lithoautotrophic metabolism involving energy generation and growth based on oxidation of reduced metals and S associated with local rock and ground waters coupled to O₂ respiration.”

A4.2. “The diversity of microorganisms associated with groundwater will be low and dominated by slow-growing oligotrophic chemoheterotrophic prokaryotes that can utilize very low concentrations of dissolved organic compounds associated with surface-derived waters. The diversity of prokaryotes associated with older, potentially geothermal water, will also be low but metabolisms will be distinct and will include organisms that can utilize dissolved gases such as H₂, CH₄, or CO as energy sources.”

A5. “The overall genetic (and phylogenetic) diversity of indigenous microbes associated with ancient (tens to thousands of Kyr.) ground water will be similar to that found in surface environments where the primary energy source is sunlight. The genes and pathways for energy metabolism and biosynthesis will also be similar. A core set of genes should also be present, however, that will allow for growth and survival under low nutrient conditions utilizing reduced gases for energy (J.K. Fredrickson, Pacific Northwest National Laboratory).”

Based on previous, although somewhat limited, knowledge the overall phylogenetic diversity of subsurface microbial communities is no greater or less than in other environments. However, adaptations that allow for function and survival in the deep subsurface should be reflected in abundant unique genes that will initially be characterized as unique hypothetical genes based on comparative DNA sequence analysis. Comparative genomic approaches can be used at the organism or community levels to test these hypotheses. Functional genomics, however, will ultimately be required to unravel the role of these unique, “deep subsurface” genes. For example, because the concentrations of carbon and energy sources will be low, the microbiota

would be expected to have highly adapted systems for assimilating nutrients at such concentrations, i.e., enzymes with low K_m values.

B. Subsurface biogeochemical processes

Subsurface biogeochemical processes emphasizes the role of microorganisms in the dissolution and precipitation of mineral phases, the transport /transformation of chemical species, and the alteration of hydrological properties (i.e. storage capacity and permeability) of aquifers. This information has vital applications to deep carbon sequestration and nuclear and non-nuclear waste disposal and stabilization. Experimentation can provide direct measurement of the release rates of CO_2 from the injection zones or of entrapment in the formation, of the chemical form and mobility of waste, and the survival of microorganisms in different thermal and chemical regimes that may be imposed by borehole injection or repository conditions. Some of the hypotheses to be investigated include those below.

B1. “Microbiologically-induced calcite precipitation contributes to mineralization in subsurface environments. Common soil microbes (e.g., *Bacillus pasteurii*) participate in CO_2 sequestration in alkaline environments. It is likely that subsurface microbial communities will contribute to CO_2 sequestration in deep subsurface environments (S. Bang, South Dakota School of Mining and Technology)”

Many anaerobic microbial processes lead to substantial increases in the pH of the environment, especially NH_3 production (Bachmeier et al., 2002) and Fe(III) and sulfate reduction, respectively. This elevation of the pH is essential in order to transform dissolved CO_2 pumped into a subsurface aquifer into more soluble HCO_3^- and CO_3^{2-} species that lead to the permanent sequestration of CO_2 as solid phase carbonate (e.g. calcite or siderite). The microbial activities that occur at the interface of supercritical CO_2 and saline groundwater can be directly examined at NUSL-OSG using instrumented fracture zones maintained. Supercritical CO_2 can be leaked into the fracture system at ambient formation pressure and its concentration, the speciation and the resulting chemical reactions monitored downgradient of the injection under a range of microbial redox conditions. This approach will provide insight into the storage capacity of a subsurface environment for CO_2 and whether any environmentally hazardous outcomes result from such a mitigation approach. These experiments will also have enormous ramifications for any postulated subsurface microbial environments on Mars where liquid to supercritical CO_2 has recently been postulated to exist (Hoffman, 2001) and on the importance of dissolved inorganic carbon to the sustenance of autotrophic communities (Stevens and McKinley, 1995).

B2. “In deep subsurface environments where gas and water occur as separate phases, microbial communities may be concentrated at the interface between gas and water where the flux of dissolved gases into the water is the greatest. (T. Onstott, Princeton University)”

Investigations into subsurface communities to date have focused upon the relationship of microbial communities and the mineral-liquid interface. In natural gas reservoirs and in some of the Au mines of South Africa, both gas and liquid are present as distinct phases. The gas constituents, typically composed of methane, light hydrocarbons and H₂, are all excellent electron donors when dissolved in water, which in turn contains electron acceptors. As microorganisms consume these electron donors, they are replenished by diffusion from the gas phase resulting in a concentration gradient. Although such an environment is easy to conceive, observing such a system is virtually impossible without having access to a fluid filled fracture that is or can be partially dewatered. OSG may provide an opportunity to study the geochemistry and microbiology of such fracture systems. To test this hypothesis requires either detailed spatial and temporal sampling of a gas-water fracture zone or experimental simulation of a gas-water system utilizing a high-pressure manifold.

B3. "The composition of the indigenous microbial community will reflect the geochemical properties, in particular the dominant electron acceptor, of the hydrogeological regime. (T. Onstott, Princeton University)"

In previously published investigations of the subsurface communities in hydrocarbon reservoirs and in reports from deep subsurface communities in the Au mines of South Africa and Aspo in Sweden, the environments are typically rich in electron donors, particularly hydrogen, but limited by the bioavailability of electron acceptors, e.g. nitrate, Fe(III), sulfate or CO₂. Depending upon where OSG is cited, the rock geochemical heterogeneity could greatly influence to composition of the microbial community. In the Fe sulfide rich portions of the formation, Fe(II) and S can act as a potent electron donors leading to the in situ formation of Fe(III) weathering products. Where these oxidized products are abundant, Fe(III) reducing bacteria should be the dominant component of the microbial community. Where the reduced Fe(II) carbonate strata exist, CO₂ reducers or autotrophic microorganisms may dominate.

C. Subsurface microbial ecology

Subsurface microbial ecology explores the evolution and adaptation of life in the deep subsurface; the relationships between microbial community structure and function and the sources of nutrients/energy that allow them to survive and reproduce, in some cases, independently of photosynthesis; identification of the subsurface chemical, geological and hydrodynamic properties that contribute to the sustenance, migration, adaptation and evolution of life in the deep subsurface and beyond. Importantly, these studies will provide an understanding with which to approach investigations of life on other planets and perhaps extra-terrestrial environments. Some of the hypotheses to be investigated include those below.

C1. "The rate of fluid flux through subsurface fracture networks should dictate the quantity and type of microorganisms present. High fluid fluxes should lead to greater biomass. Variable fluid fluxes should create greater diversity. (F. Colwell, Idaho National Engineering and Environmental Laboratory)"

Fluid flux is believed to be a key component of whether microbial cells flourish or perish in the subsurface. Electron donors and acceptors are required for energy conservation. Without some movement through the fracture system, however, life will only exist proximal to those sources of energy for a brief moment in geological time, before life reduces the chemical free energy to zero. This hypothesis could be tested at the OSG using two approaches. The first approach is to combine measures of microbial biomass and diversity on samples of groundwater and rock cores adjacent to fractures with noble gas isotopic analyses. In fractures where fluid migration rates are rapid (~1 m/yr), the noble gas isotopic composition of the fracture fluid will be distinct from that of the rock's pore water. In fractures where fluid migration rates are extremely slow (~1 mm/yr) the noble gas isotopic composition of the fracture fluid will be similar to that of the rock's pore water. The second approach is to install into a borehole-intersected fracture zone a device that allows: 1) regulation of the rate of flow through the fracture zone and thus the flux of groundwater substrates and 2) insertion of some colonizable and retrievable substrates that can be evaluated over time to determine the relationship between flux rate and biomass and diversity.

C1.1. "Different genes should be expressed by subsurface microbial communities in fractures depending upon the rate at which fluids pass over the communities. (F. Colwell, Idaho National Engineering and Environmental Laboratory)"

Because the fluid flux will control the relative concentrations of energy and growth substrates and toxic species in the fracture environment, existing subsurface microbial communities must have metabolically adapted to this nutrient flux, otherwise they would have expired. One approach to observing these adaptations under forced flow conditions is monitor the expression of genes responsible for specific electron transport pathways.

C2. "Spontaneous mutagenesis will be less in subsurface environments shielded from cosmic rays and low in radiogenically generated gamma radiation than in soil zones. (P. Zimmerman, South Dakota School of Mining and Technology)"

The rate of microbial evolution in the subsurface is affected by growth rates, spontaneous mutagenesis, the rate of genetic exchange and the rate at which the environment changes. Growth rates in the subsurface are much less than those in surface marine and terrestrial environments and hence the rate of DNA repair or propagation of altered DNA is also much lower. Depending upon the rate of spontaneous mutagenesis by radiation, mutagenesis by DNA damage and growth may not be the principal mechanism for evolution of subsurface microorganisms. OSG offers an opportunity to study the effects of ambient radiation on mutagenesis in an environment in which the radiation flux and spectra are extremely well established. Mutation rates could be quantified by utilizing the; 1) Ames test, which uses an auxotrophic mutant strain of *Salmonella typhimurium* and test for back mutations in a single gene, and 2) bacteria for which the entire genome has been sequenced. These bacterial dosimeters can be situated at different depths within NUSL and their spontaneous mutagenic rate compared as a function of ambient radiation.

C3. “In ancient, deep subsurface groundwater environments where electron donors are abundant, but electron acceptors are limited microorganisms will either possess metabolic plasticity to utilize several electron acceptors and or live synergistically with other microorganisms that consume or cycle their waste products. (T. Kieft, New Mexico Institute of Mining and Technology)”

Stevens and McKinley (1997) have observed that in the H₂ rich subsurface environment of the Columbia River Basaltic Aquifer, that acetogens (utilizing H₂ and CO₂) generate acetate and postulate that this acetate may be utilized by heterotrophic microorganisms, such as acetate oxidizing sulfate reducers, to regenerate the CO₂. The syntrophic relationship ensures that the acetate concentrations never approach the level that shut down the autotrophic activity of the acetogens. If ancient, geohydrologically isolated fluid filled fractures exist at OSG, then the microorganisms they contain are likely to have been isolated from the surface for millions of years. By comparing the microbial communities and geochemistry of these environments with those found in fractures where the fluid is being more directly recharged from the surface will determine to what extent deep subsurface microbial communities are dependent upon transport of life-sustaining substrates from the surface. Such a comparison will also reveal what adaptations these microorganisms have made for extremely long-term survival.

C4. “The large scale transport of shallow subsurface or soil microorganisms to the terrigenous deep subsurface through fracture zones will be more limited by their ability to survive and grow in the deep-subsurface rather than by their transport properties. (T. Kieft, New Mexico Institute of Mining and Technology)”

The concentration of microorganisms in soils are many orders of magnitude greater than that of solid rocks in the deep subsurface; whereas, the concentration of microorganisms in shallow groundwater is only two orders of magnitude greater (Whitman et al. 1998; Onstott et al., 1998). Are soil microbes ultimately the feedstock for subsurface microbial communities? In the absence of a dynamic, hydrothermal fluid system, e.g. deep-sea vents, fluid velocities in the terrigenous crust are typically very slow. At such velocities, the adhesion of microorganisms to solid mineral surfaces are so high that the planktonic cell density of downward migrating soil microbes would be reduced by three orders of magnitude long before they have reached the deep subsurface. Consequently, only those surface microorganisms that can readily grow in the anaerobic, nutrient limited, higher temperature environments will ultimately appear as a member in the subsurface community. Those that do not grow will be quickly winnowed from the water mass by adsorption to the rock matrix. Two approaches can be followed to examine this hypothesis. The first is to document the microbial community structure as a function of depth along a fracture system that is demonstrably connected to the surface. If soil microorganisms are penetrating over a kilometer into the crust, their characteristic 16S rDNA signature should be found in the subsurface communities. A second approach is to instrument several well-characterized fracture zones, OSG will be the only facility in the world to be able to perform microbial transport experiments in a fractured rock

system over a depth range of kilometers. Using the same instrumentation described above for fluid flux experiments, surface organisms collected at OSG can be injected into the fracture zones at depth and their transport and growth monitored relative to that of subsurface microorganisms adapted to the fracture environment.

C5. “The base of the subsurface biosphere is currently pinned at 120°C, the higher survival temperature known for a microorganism in the lab. The upper temperature limit of life in the subsurface however is controlled by a balance between the energy available for hyperthermophiles to repair their cell membranes, enzymes and DNA and the thermal stability of their membranes, enzymes and DNA. This may mean that unlike the laboratory experiments and the deep-sea vents where nutrient fluxes are very high, the upper temperature limit for life in the terrigenous subsurface is much less than 120°C. (T. Onstott, Princeton University)“

From a small drilling platform located at the deepest level of OSG vertical coring to depths for which the formation temperature is 120°C, the highest temperature limit for known life forms, is feasible and economical. By undertaking a small coring program, OSG could provide the first unequivocal evidence for the upper temperature limit of life in the deep subsurface. Once completed the packered borehole could be utilized to test the survivability of known hyperthermophiles in this oligotrophic environment by submerging them into the borehole on filter enclosed, rock chip coupons.

EDUCATIONAL OBJECTIVES

OSG will be involved in international, national, regional and local educational outreach at all levels from casual site visitors to K-12 to graduate and post graduate and through to senior scientists. Outreach programs will include the following:

1. OSG would be responsible for the biological component to a scientific visitors' center at OSG.
2. Secondary school workshops focusing on local schools, and real time instruction aides over the Internet for regional schools with lesson plans for K-12.
3. Secondary school teacher training workshops focusing on local schools and supplied with educational aides and video tours of excursions.
4. Deployment of experiments designed by local and regional high school students and that can be monitored real time through the Internet. The International/High Plains Regional Science and Engineering Fair provides an ideal venue for organizing the student participation and the mentoring experience by their teachers. Experiments could include the insertion of media-bearing cartridges designed by the students into boreholes along with pH, Eh, O₂ sensors connected to short-term memory storage at the borehole head that can be periodically downloaded through the Internet. The media cartridges can be removed for analyses at the high school or electron microscopic observations at EMES with images displayed real time on the Internet.
5. Summer field research institute-training-local, national, and international college students and senior scientists-workshop modeled after the summer program of

the Marine Biological Lab (MBL) at Woods Hole and the recent minority, educational workshop recently held in South Africa <http://web.utk.edu/~kdavis>.

6. OSG will host an REU site for undergraduates nationwide and targeting minority groups. This could be coordinated with the pending REU for South Africa where students have an opportunity to attend both and including excursions and experiments examining biogeochemical processes of samples.
7. OSG will target the minority communities at the secondary school and college level and internships, undergrad, grad, postmasters, postdoctoral programs.
8. Undergraduate and graduate students funded through NSF's IGERT Program at Oregon State University and Portland State University (Subsurface Biosphere Interdisciplinary Doctoral Program) could conduct research at NUSL-OSG.
9. OSG could host visiting scientists intent on performing underground experiments and conferences for such societies as the ISSM (International Society for Subsurface Microbiology) and the ISE (International Society for Extremophiles).

IMPLEMENTATION

During the initial 3 to 5 years, the primary focus of the geomicrobiological effort at OSG will be on examining the biological diversity of the deep subsurface microbial at OSG and establishing an educational and outreach program. This requires gathering observational and experimental data over a multi-year time scale from a variety of subsurface settings. The early establishment of a OSG that coordinates this multidisciplinary research effort with educational objectives is essential to its successful implementation. OSG should be comprised of a surface facility with laboratories and support personnel and subsurface experimental facilities at several localities where the migration, diversity and metabolic activity of microbial communities can be monitored over extended time periods. More sophisticated laboratories and supervising faculty can be cited at the local universities (see Appendix 1 as an example of facilities available), complemented by affiliated national and international institutions where highly specialized measurements (e.g. ion probe and accelerator mass spectrometer analyses) can be performed.

The OSG will be the only facility of its kind where long-term experiments can be performed by the international community in fractured, heterogeneous, metamorphic rock with manned infrastructure at depths up to 2.7 kilometers and with deeper flow fields, boreholes and screened wells extending beyond 4 km beneath the surface. Microbiological observations and experiments conducted at OSG will build upon 15 years of research investment in subsurface biogeochemistry sponsored by the Department of Energy's Subsurface Science Program and the NSF's Life in Extreme Environments (LEn) program, now both defunct. The data gathered from this lab will complement observations that have been reported over the last 10 years from the shallower 400-meter deep Äspo Underground Laboratory in Sweden, where experiments have just recently begun in underground facilities hosted by a fractured, homogenous, granite aquifer. Long term observations made at OSG can also be compared to shorter-term data gathered in active South African mines, where samples collected from depths as great as 3.5 kilometers have been obtained. Long term experiments performed at OSG could address many questions raised by the observations made in South African

mines and in boreholes or mines around the world and could be designed to test the microbial impact on subsurface CO₂ sequestration and underground radioactive waste disposal. The OSG could also provide an invaluable test bed and training ground for and opportunity for technology transfer to NASA engineers attempting to develop methods for detecting life beneath the surface of Mars. Finally, NUSL-OSG could tap into a new gene pool with potentially valuable biotechnological applications by fostering interactions and CRADA's with biotechnology and pharmaceutical firms.

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