

Potential Microbial Applications

Researchers have only scratched the surface of microbial biodiversity. The earth's biomass is largely microbial, but most of these microbes are unknown and uncharacterized. Sequencing the genomes of microbes provides insights into how they survive and manipulate their environments; it affords opportunities to exploit biochemical mechanisms and pathways not expressed in higher organisms. Knowledge about the enormous range of microbial capacities is gained at a fundamental level, with broad and far-reaching implications for environmental, energy, health, and industrial applications.

- Cleanup of toxic-waste sites worldwide. Alter bacterial genomes to enhance their usefulness, perhaps by adding genes from other organisms to degrade toxic chemicals such as toluene or alter the chemical state of toxic metals commonly found in mixed, chemical, and radiological waste sites.
- Production of novel therapeutic and preventive agents and mechanisms. Develop microbes that produce pharmaceuticals like those now supplying human insulin and growth hormone.
- Energy generation and development of renewable energy sources, e.g., methane and hydrogen.
- Chemical catalysts, reagents, and enzymes to improve the efficiency of industrial processes.
- Management of environmental carbon dioxide, which is related to climate change.
- Detection of disease-causing organisms and monitoring of the safety of food and water supplies. Develop diagnostic systems for identifying pathogens to counteract biological warfare threats.
- Use of genetically altered bacteria as living sensors (biosensors) to detect harmful chemicals in soil, air, and water. Some altered bacteria emit a green fluorescent protein visible in UV light when they metabolize the explosive TNT leaking from land mines. Others might be applied to a tract of land by crop dusting for later analysis of pollutants.
- Understanding of specialized, shared systems used by disease-causing organisms (pathogens) to disable or destroy human cells. Use insights gained to learn how pathogens infiltrate organisms with protein-coding genes capable of sneaking past human defense systems. Such protein structures may provide ideal targets for developing completely new types of antibiotics.

Related Research

The Microbial Genome Project (MGP) is developing and improving tools and methods for studying how genes work. Called **functional genomics**, this field:

- studies how genes interact to produce specific products or influence particular behaviors,
- analyzes genomic information from sequenced microbes to obtain insights into their particular characteristics,
- improves tools for annotation and analysis of sequence data, and
- develops methods for determining gene function and gene expression and for examining protein-protein and protein-nucleic acid interaction.

As part of DOE's Biological and Environmental Research (BER) Program, MGP research is relevant to BER's **Natural and Accelerated Bioremediation Research** (NABIR) Program. NABIR's goal is to provide the scientific understanding needed to harness and develop methods to accelerate natural microbial processes for the bioremediation of contaminated soils, sediments, and groundwater.

The success of the MGP has led to initiation of the **Microbial Cell Project**. Launched in FY 2001, it will integrate data from the MGP with other genomic research to lay a foundation for understanding how cellular components function together to create living systems.

A revolutionary new BER program, called "**Genomes to Life**," expands the scope of the MCP by using completed microbial (and human) DNA sequences as a starting point. Researchers will combine the sequences with new high-throughput technologies to develop a set of comprehensive principles and models of how living systems function. The goal is to systematically tackle questions about the composition and function of the cellular components that carry out and control critical life processes. **Genomes to Life** is geared particularly to systems with capabilities of interest to DOE.

Related Web Sites

Carbon Sequestration Program
cdiac2.esd.ornl.gov

Genome Annotation Data
<http://genome.ornl.gov/microbial>

Genomes to Life
<http://DOEGenomesToLife.org>

Joint Genome Institute Microbial Sequencing
www.jgi.doe.gov/tempweb/JGI_microbial/html/index.html

Microbial Cell Project
<http://microbialcellproject.org>

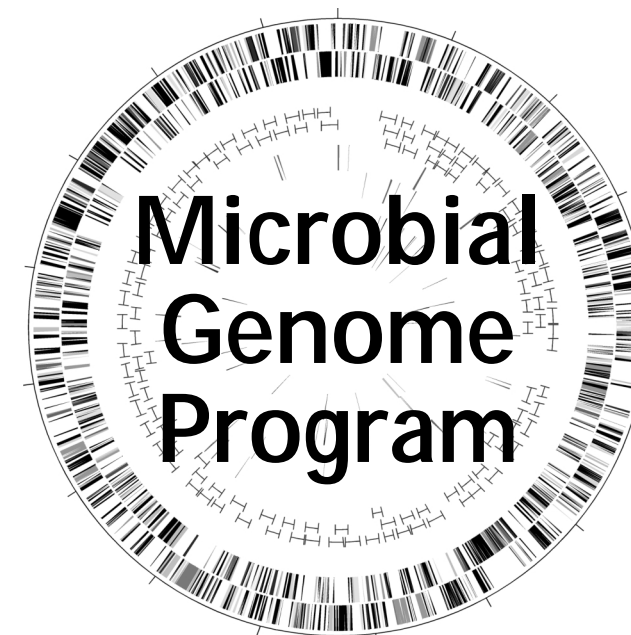
Natural and Accelerated Bioremediation Research
www.lbl.gov/NABIR

The Institute for Genomic Research Microbial Database
www.tigr.org

For more information on the Microbial Genome Program and related programs, visit the Web sites listed above or contact Daniel Drell. To request print copies of the *Microbial Genome Report* or other publications, contact HGMIS.

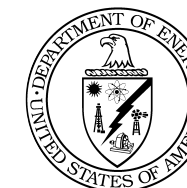
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Sequencing and exploiting entire genomes of microorganisms having potential usefulness in energy, environmental, health, industrial, and microbial-diversity research

**U.S. Department of Energy
Office of Biological and
Environmental Research**



www.science.doe.gov/ober/microbial.html
www.ornl.gov/microbialgenomes

Microbial Genome Program

U.S. Department of Energy Office of Biological and Environmental Research

The U.S. Department of Energy (DOE) supports innovative high-impact and peer-reviewed science. Its missions include a range of such difficult challenges as environmental-waste cleanup, energy production, carbon sequestration, and biotechnology. To aid in carrying out its missions, DOE initiated the Microbial Genome Program (MGP) in late 1994 as a spinoff of its Human Genome Program. Scientists expect to find a vast repertoire of useful functions in the microbial world that could be applied to solving challenges in the human world (see back pages).

The MGP aids DOE in carrying out its established missions by generating novel insights into both the biological underpinnings of climate change and the role of microbes in the overall processing of metals, carbon, radionuclides, and nitrogen on earth. Scientists are only beginning to appreciate the role of microbial life in, for instance, global climate processes.

The MGP represents yet another example of DOE's overall aim to support bold research that applies interdisciplinary science, an element of intellectual risk, and revolutionary solutions to DOE's energy-related biological and environmental challenges.

Why Microbes?

Microbes, which make up most of the earth's biomass, have evolved for some 3.7 billion years. They have been found in virtually every environment, surviving and thriving in extremes of heat, cold, radiation, pressure, salt, acidity, and darkness. Often in these environments, no other forms of life are found and the only nutrients come from inorganic matter. The diversity and range of their environmental adaptations indicate that microbes long ago "solved" many problems for which scientists are still actively seeking solutions.

The future promises many exciting developments as the fruits of the Microbial Genome Program are harvested. We are becoming more appreciative of the microbial world's profound effects on earth and its potential benefits.

Sequencing Microbial Genomes to Uncover Potential Applications Relevant to DOE Missions

Carbon Sequestration

***Chlorobium tepidum* (bacteria, 2.1 Mb): Photosynthetic; may play important role in earth's overall carbon cycle.

†*Chloroflexus aurantiacus* (bacteria, 5 Mb): Modern version of organism that needs no oxygen for photosynthesis. Uses unique pathway to fix carbon dioxide.

Nitrosomonas europaea (bacteria, 2.8 Mb): Important in soil nitrogen cycling and ammonia oxidation; degrades chlorinated hydrocarbons; aids incorporation of carbon dioxide into biomass.

Nostoc punctiforme (bacteria, ~9 Mb): Fixes carbon dioxide and nitrogen; produces hydrogen; survives acidic, anaerobic, and low-temperature conditions.

***Prochlorococcus marinus MED4* (bacteria, 1.7 Mb) and *Prochlorococcus marinus MIT9313* (bacteria, ~2.3 Mb): Both abundant in temperate and tropical oceans; important in ocean carbon cycling; absorb blue light efficiently. MIT9313 is adapted to lower-light conditions (lower ocean depths).

***Rhodospseudomonas palustris* (bacteria, 5.5 Mb): Fixes carbon dioxide; produces hydrogen; biodegrades organic pollutants under both aerobic and anaerobic conditions.

Synechococcus WH8102 (bacteria, 2.4 Mb): Photosynthetic; important to ocean carbon fixation; genetically tractable.

Thalassiosira pseudonana (eukarya, ~25 Mb): Ocean diatom, major participant in biological "pumping" of carbon to ocean depths.

Energy Production

**Methanobacterium thermoautotrophicum* (archaea, 1.7 Mb): Produces methane; plays role in earth's overall carbon cycle.

**Methanococcus jannaschii* (archaea extremophile, 1.7 Mb): May identify high-temperature, high-pressure enzymes; produces methane.

†*Methanosarcina barkeri fusaro* (archaea, 2.5 Mb): Lives in cattle rumen; digests cellulose and other polysaccharides to produce methane. Very oxygen sensitive. Grows in variety of substrates.

Methylococcus capsulatus (bacteria, 4.6 Mb): Uses methane as single carbon and energy source; generates pollutant-oxidizing enzymes; used commercially to produce biomass and other proteins.

Bioremediation

†*Burkholderia cepacia LB400* (bacteria, 8 Mb): Outstanding degrader of polychlorinated biphenyls (PCBs).

***Caulobacter crescentus* (bacteria, 3.8 Mb): Potential for heavy-metal remediation in waste-treatment plant wastewater.

Dehalococcoides ethenogenes (bacteria, less than 2 Mb): Degrades dangerous solvent trichloroethene to benign products.

**Deinococcus radiodurans* (bacteria, 3 Mb): Survives extremely high levels of radiation; possesses DNA-repair capabilities for radioactive waste cleanup.

†*Desulfitobacterium hafniense DCB2* (bacteria, 4.6 Mb): Degrades pollutants such as chlorinated organic compounds that include some pesticides.

Desulfovibrio vulgaris (bacteria, 1.7 Mb): High potential for bioremediation through metal and sulfate reduction and sulfate utilization.

†*Ferroplasma acidarmanus* (archaea, ~2.8 Mb): Lives in most acidic conditions on earth. Oxidizes iron. Transforms sulfide in metal ores to sulfuric acid, leading to contamination of mining sites.

Geobacter sulfurreducens (bacteria, 2.9 Mb): Reduces a variety of metals, including iron and uranium.

†*Magnetospirillum magnetotacticum MS-1* (bacteria, 4.5 Mb): Requires limited oxygen. Reduces iron, produces magnetite. Possible model for biomineralization and evolutionary responses. May serve as a geomagnetic tracer.

†*Pseudomonas fluorescens PFO-2* (bacteria, 5.5 Mb): Metabolically diverse. Degrades pollutants such as styrene, TNT, and polycyclic aromatic hydrocarbons. Useful in applications requiring bacteria release and survival in soil.

Pseudomonas putida (bacteria, 5 Mb): High potential for bioremediation by reducing metal and pollutants.

†*Ralstonia metallidurans CH34*, formerly *Ralstonia eutropha* and *Alcaligenes eutrophus* (bacteria, ~5 Mb): Contains two "mega" plasmids. Resistant to wide variety of heavy metals, which accumulate on the cell surface. Strong potential for bioremediation of metals.

†*Rhodobacter sphaeroides 2.4.1* (bacteria, 4.4 Mb): Metabolically diverse, grows in wide variety of conditions. Photosynthetic. Provides fundamental insights into light-driven, renewable-energy production. Can detoxify metal oxides, useful in bioremediation.

Shewanella oneidensis (bacteria, 4.5 Mb): May degrade organic wastes and reduce or sequester a range of toxic metals.

†*Sphingomonas aromaticivorans F199* (bacteria, 3.8 Mb): Degrades aromatic compounds in soil, including toluene, xylene, naphthalene, and fluorine.

Thiobacillus ferrooxidans (bacteria, 2.9 Mb): Used in mining industry to sequester iron and sulfide.

Cellulose Degradation

†*Clostridium thermocellum ATCC27405* (bacteria, ~5 Mb): Thermophilic; degrades cellulose via multienzyme complexes ("cellulosomes").

†*Cytophaga hutchinsonii* (bacteria, ~5 Mb): Very abundant in nature; decomposes cellulose, lacks cellulosomes.

Phanerochaete chrysosporium (eukarya, ~20 to 30 Mb): "White rot" fungus; aerobic and degrades both celluloses and lignins; can also degrade polyaromatic hydrocarbons.

†*Thermobifida fusca*, formerly *Thermomonaspora fusca* (bacteria, 8 Mb): Major degrader of organic materials, responsible for Farmer's Lung disease.

Industrial Processes

**Aquifex aeolicus* (bacteria extremophile, 1.5 Mb): Potential for identifying high-temperature enzymes.

**Archaeoglobus fulgidus* (archaea extremophile, 2.3 Mb): Potential for identifying high-temperature and high-pressure enzymes; useful in oil industry.

***Clostridium acetobutylicum* (bacteria, 4.1 Mb): Produces acetone, butanol, and ethanol; useful for industrial enzymology.

**Halobacterium halobium* plasmid (archaea, 2.3 Mb): Potential for identifying high-salinity enzymes.

†*Magnetococcus MC-1* (bacteria, 4.5 Mb): Requires limited oxygen. Reduces iron. Produces magnetite, which has many practical commercial uses.

***Pyrobaculum aerophilum* (archaea extremophile, 1.8 Mb): May identify high-temperature enzymes.

***Pyrococcus furiosus* (archaea extremophile, 2.1 Mb): May identify high-temperature enzymes.

**Thermotoga maritima* (bacteria extremophile, 1.8 Mb): Potential for identifying high-temperature, high-pressure enzymes. Metabolizes many simple and complex carbohydrates; possible sources of renewable carbon and energy.

Technology Development, Pilot Projects

**Borrelia burgdorferi* (bacteria, 1.3 Mb): Human pathogen that causes Lyme disease. One linear chromosome (915 kb) supported by DOE. Entire genome published by TIGR.

**Mycoplasma genitalium* (bacteria, 580 kb): Human pathogen; serves as model for minimal set of genes sufficient for free living.

†*Xylella fastidiosa (almond)* (bacteria, 2.8 Mb): Pathogenic to economically important plants such as orange and almond trees.

†*Xylella fastidiosa (oleander)* (bacteria, 2.8 Mb): Pathogenic to plants, particularly oleanders.

†Draft sequence by the DOE Joint Genome Institute.

*Completed and published (see www.tigr.org/tdb).

**Completed, not published (as of February 2001).