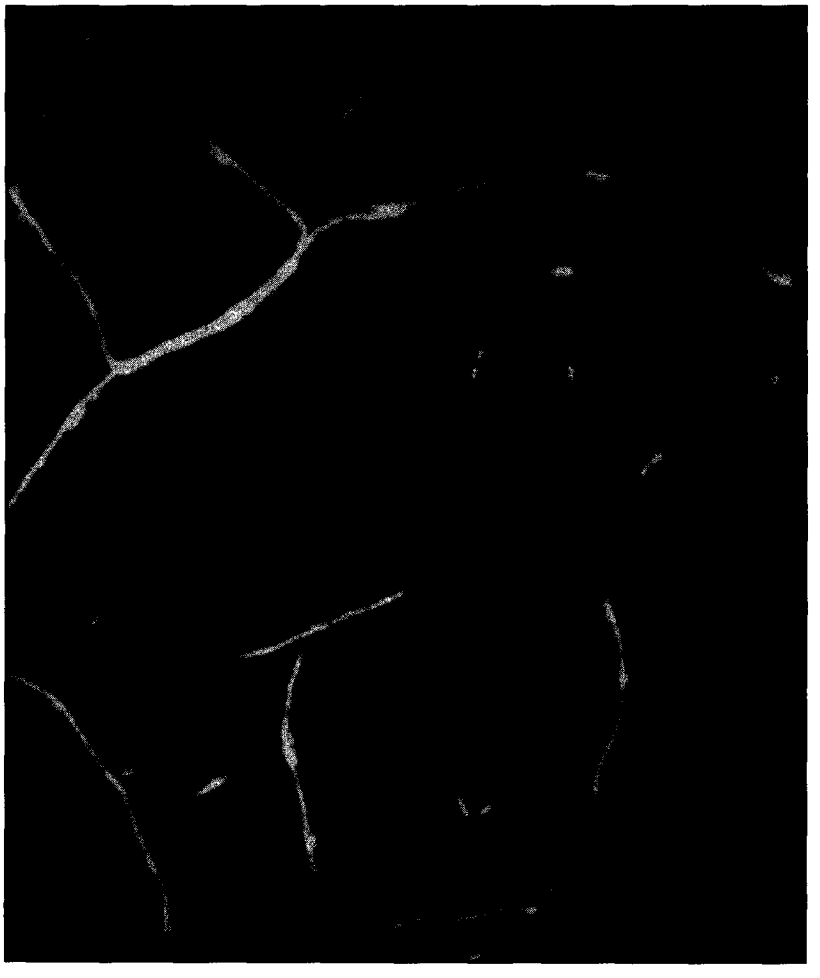
United States
Environmental Protection
Agency

Office of
Research and Development
Washington DC 20460

EPA 600/K-93/007
January 1994

EPA Biotechnology

Research Program Overview



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Cover Photo: Micro autoradiogram of acridine orange stained *Pseudomonas aeruginosa* PAOI viewed under blue light excitation. Many cells exhibit a metabolically active, but non-reproducing filamentous morphology as a result of incorporation of *E. coli* lethal genes.

Contents

EPA's Biotechnology Research Program, begun in 1984, focuses on predictive assessment of microbial biotechnology products. This booklet describes the various types of research, and how they contribute information needed for assessment and regulation of these products.

EPA'S ROLE IN BIOTECHNOLOGY

The use of living organisms (or parts of organisms) to make or modify products, to improve plants or animals, or to develop microorganisms for specific uses forms the basis of ***biotechnology.***[1] The tremendous potential of this technology encompasses the culturing of microorganisms for toxic chemical degradation and production of foods and pharmaceuticals; the creation of rapid, sensitive diagnositic medical tools; and safe, effective control of agricultural pests.

The promise offered by such developments is evidenced by the growth of the biotechnology industry. The industry is said to be "still inventing itself"[2], as it has grown from under 100 companies in 1970 to over 1,000 companies today.

Goals of the Risk Assessment Research Program

To provide the Agency with the scientific tools to:

- Allow beneficial uses of the technologies to move forward in a safe manner.
- Accurately assess the risk of biotechnology products.
- Successfully control the use of products where warranted.

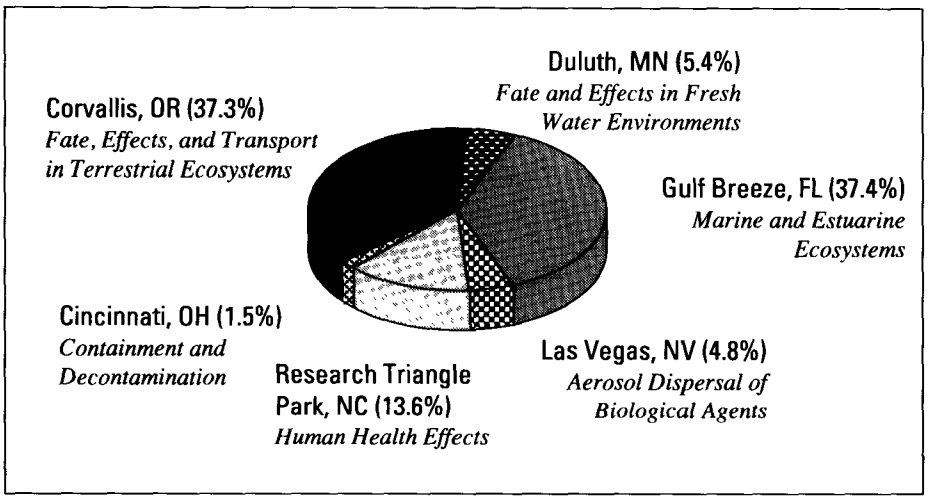
"Given the growing societal focus on environmental issues and increased awareness of environmental threats, the potential contributions of environmental biotechnology may be far greater than were earlier anticipated."

-from the Ernst & Young Report Biotech 91: A Changing Environment[2]

[1]*U.S. Congress, Office of Technology Assessment, Oct., 1991. Biotechnology in a Global Economy, OTA-BA-494, U.S. Government Printing Office, Washington, D.C.*

[2]*Burrill, S.G., and K.B. Lee, Jr. 1990. Biotech 91: A Changing Environment. Ernst & Young, San Franscico, CA., pages 1 & 4.*

In order to ensure appropriate regulatory oversight of the biotechnology industry[3], without interfering with the safe development and commercial use of biotechnology products, the U.S. Environmental Protection Agency (EPA) is developing procedures to assess the potential risks that may be associated with the manufacture, distribution, and use of microbial products. The EPA Office of Research and Development (ORD) administers a research program structured to provide the scientific tools for this risk assessment process; tools that allow sound evaluations of the safety of various uses of bacteria, fungi, and viruses, including certain genetically modified microorganisms.

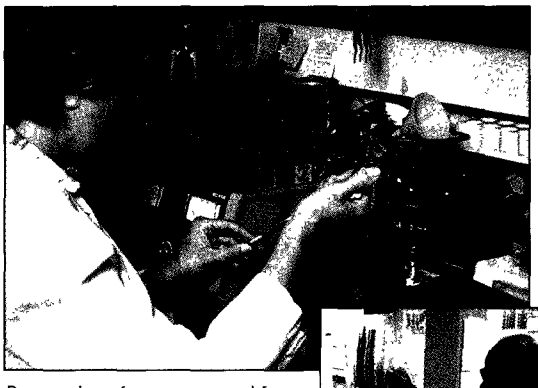


Relative participation of various EPA/ORD Laboratories in the Biotechnology Research Program based on FY 1992 funding levels.

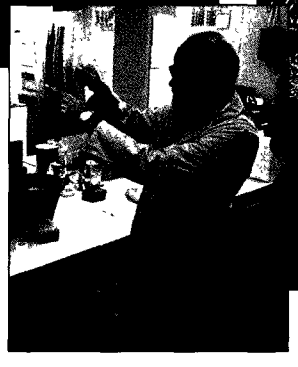
[3]*The U.S. Environmental Protection Agency functions under a number of statutes to carry out its mission of protecting human health and the environment. While these statutes were not written specifically to regulate biotechnology, there are two which have been interpreted as investing EPA with the authority to do so: the Toxic Substances Control Act (TSCA) and the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). Under the Coordinated Framework for Regulation of Biotechnology, EPA regulates many uses of microorganisms (see p. 8); the U.S. Department of Agriculture (USDA) regulates agricultural uses of microbes, plants, and animals; and the Food and Drug Administration (FDA) regulates foods, drugs, cosmetics, and biologics.*

STAFF

The EPA scientific staff combines expertise in biotechnology and risk assessment procedures to bring a unique scientific focus to the research program. They are building the information base and developing appropriate methods and protocols for risk assessment. The staff scientists also foster interactive information exchange with scientists in the fields of genetics, biochemistry, ecology, and microbiology. They share responsibility for a complimentary extramural program administered through cooperative agreements, interagency agreements, and contracts. Regular, independent reviews of the research program serve to guide the focus toward the needs of the regulatory offices of EPA while maintaining a high standard of scientific quality.



Preparation of an agarose gel for analysis/recovery of DNA restriction fragments.



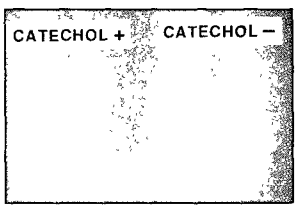
Researchers performing DNA sequence analysis to track bacteria in the environment

Sampling zooplankton from a temporary pool site prior to application of the mosquito control microorganism, *Bacillus thuringiensis* var *israelensis*

THE BIOTECHNOLOGY RESEARCH PROGRAM

The Research Program's activities are divided into three categories that reflect those areas of research considered crucial to EPA's regulatory needs. These categories are: A. Environmental Exposure Studies, B. Environmental Effects Studies, and C. Control Strategies.

A. Environmental Exposure Studies: *aimed at understanding how microorganisms survive, move, and interact in various environments*

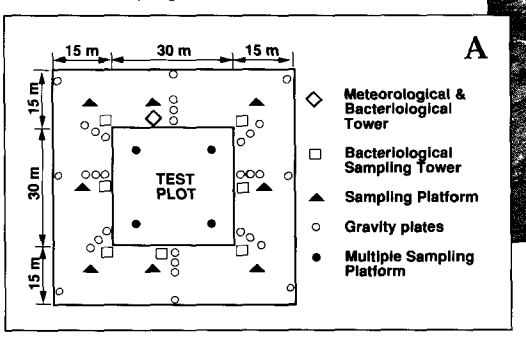


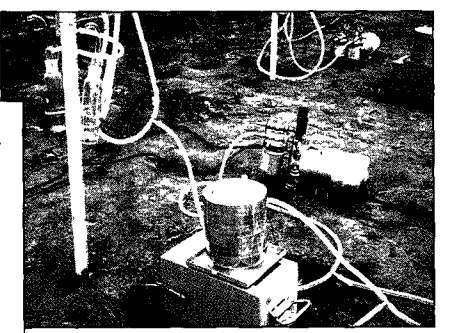
Pigment production triggered by catechol represents a quick and convenient means to enumerate or verify the identity of certain microorganisms in natural environments

Detection and Enumeration

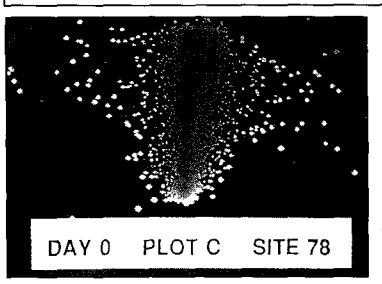
This work focuses on improving methods of identifying and measuring populations of microorganisms. Innovative detection and analytical methods are being developed and assessed for their applicability and potential usefulness in the risk assessment process. Microbial, serological, biochemical, and genetic methods are being tested and refined under laboratory conditions to maximize their sensitivity, and specificity, for microbes in soil, water, air, sewage, and animal and human digestive tracts.

Location of various samplers in a plot containing strawberries that was sprayed with ice minus *Pseudomonas syringae*





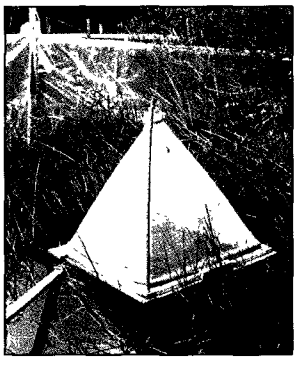
Andersen Samplers and all-glass impingers have been used to monitor survival and spread of microorganisms following aerosol releases



Surface of an agar plate recovered from a Reynier sampler used to monitor the time and concentration kinetics of *Pseudomonas syringae* applied in aerosols

Transport

An understanding of the movement of microorganisms through the environment is fundamental to evaluating the safety of biotechnology products. The mechanisms and dynamics of transport between and within various environmental components (e.g., air, soil, ground water, water, plants, insects, animals, and humans) are being studied. Where appropriate, this research may extend to the development of mathematical modeling frameworks and other decision support systems for predicting transport and consequent exposure.



Pyramid-shaped mesh cage for collection of emerging insects to determine uptake and "fly-away" of a microbial pest control agent from the site.

Survival and Colonization

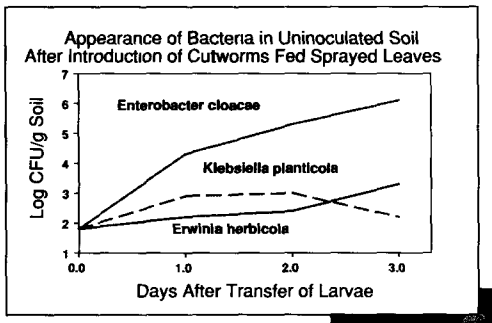
A vital question preceding commercial development and distribution of biotechnology products is, "What is the potential for microorganisms to survive and colonize particular habitats and under a variety of conditions?" The pertinent cellular properties of the microorganisms and relevant environmental factors that influence survival and colonization are being identified and described experimentally. This research necessarily builds upon detection/enumeration technologies as well as transport information. Here again, much of the effort is planned to facilitate the development of mathematical modeling frameworks and other decision support systems.

Temporary woodland pools are used to study the distribution, survival, and effects of invertebrate populations of the registered microbial pest control agent, *Bacillus thuringiensis*.

Gene Exchange

This research includes analyses of genetic exchange between microorganisms released to the environment and their indigenous counterparts. Environmental, genetic, organismal, and taxonomic factors which affect and control gene stability and the rates of gene exchange in the environment are being studied in a variety of different environmental situations.

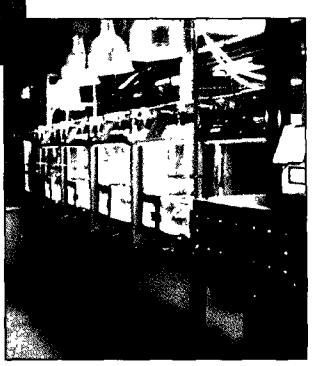
B. Environmental Effects Studies: *aimed at evaluationg the effects of biotechnology products on organisms that share their environment*



Peridroma, a leaf-eating plant pest, can be readily cultured in the laboratory, and the larvae used for conducting fate and genetic stability experiments with microbial pest control agents (MPCAs) Microcosm studies show that microorganisms can be transported by insects from sprayed plants to the soil, where they may multiply.

Ecological Processes

Environmental conditions can be simulated on a small scale in "microcosms" in the laboratory. This allows scientists to test experimental procedures. Microcosms also help to identify and describe the effects of changes in the environment on natural microbial communities and the organisms linked to them by the food web. Such effects can also be estimated through the use of computer programs designed to model the behavior of organisms in the environment.



Tiny flourescent latex particles applied in enclosed chambers are used to simulate exposure of birds to microbial pesticides Such studies are important for determining whether these pesticides pose risks to wildlife.

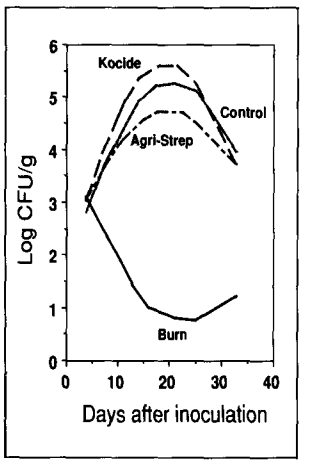
Higher Organisms

There is a wide variety of microorganisms that may be used as biotechnology products. For many, there is very limited information regarding their effects on other organisms. The focus of the current research is on determining the means (genetic and molecular) by which biotechnology products might have toxicological or pathogenic effects, and on identifying the host organisms likely to be affected. Results will be used to develop protocols for testing the effects of microorganisms on various vertebrates and invertebrates.

C. Control Strategies: *focuses on assuring containment of biotechnology products in field situations and production facilities*

Field Releases

Methods of limiting the movement of microorganisms and of monitoring them in the environment are being developed. Experiments include the actual application of microorganisms (including genetically modified microbes) to specially designed and approved experimental release sites representing different environmental habitats. Controlled conditions allow for practical evaluation of experimental techniques and assure proper containment during field releases.



Regression lines of survival of *Erwinia herbicola* in tilled plots (From Donegan, K , R. Seidler, and C Maytac. 1991. Can. J. Microbiol. 37 708-712)

Mitigation

A variety of mitigation and risk reduction strategies are being evaluated. These include novel biological controls to limit the survival and gene exchange of modified microorganisms, and physical and chemical decontamination methods for field sites. Since environmental factors affect the effectiveness of such procedures, each is being evaluated under a variety of environmental conditions.

Studies are being conducted on the persistence of microorganisms sprayed on plants, and on methods of reducing populations of these microorganisms in soil.

Process Containment

This work focuses on identifying the causes of accidental emissions of biotechnology products from production facilities, and on options for controlling emissions and reducing exposure. EPA is developing engineering and cost models for assessing risks associated with biotechnology processes in large-scale fermentation facilities. Process equipment design, decontamination technology, worker exposure and protection, and loss prevention techniques are integral components.

Use	Number of Different Microbial Species Reported In the Literature 1980 -1986
Agriculture	296
Conversion of Biomass	1,711
Industrial Chemical Production	1,058
Energy	356
Mining/Metal Recovery	167
Polymer/Macromolecule Production	1,213
Enhanced Oil Recovery	48
Waste/Pollutant Degradation	1,926
Other	133

Source: RIB Function/Organism Matrix - Fall 1986

Biotechnology Applications Under EPA Purview

- Pollutant Degradation
- Enhanced Oil Recovery
- Wastewater Treatment
- Lignin Degradation
- Bioleaching of Ores
- Nitrogen Fixation
- Fuel Desulfurization
- Biomass Conversion
- Pest Control
- Ice Nucleation (Snow making)

RESEARCH SUMMARIES

The following summaries are abstracted from Review of Progress in the Biotechnology-Microbial Pest Control Agent Risk Assessment Program, EPA/600/R-92-147, and reflect the status of EPA in-house research and research funded through cooperative agreements. Requests for proposals for extramural research are issued annually. Most cooperative agreements are funded for 2 or 3 years, and ORD initiated 32 agreements in the first 8 years of the program.

A. Environmental Exposure Studies

Perspectives on Plasmid Stability: A Study of the EPA Benchmark Plasmids, *T.T. Nguyen and R.E. Lenski, Dept. of Ecology and Evolutionary Biology, University of California*
This study focuses on the problem of plasmid loss from a population of genetically engineered microorganisms.

Studies on Conjugal Transfer of Plasmids From GEMs to Indigenous Aquatic Bacteria, *T. Barkay, U.S. EPA, Gulf Breeze, FL*
The utility of catabolic gene assembly as a tool for detection of conjugal transfer of recombinant plasmids to indigenous microorganisms was demonstrated under optimal conditions. This experimental approach will allow determinations of the effect of environmental parameters on transfer of conjugal plasmids.

Environmentally Induced Genetic Instability in Microorganisms, *T.A. Kokjohn[1], and R.V. Miller[2] ([1]Argonne National Laboratory, IL, [2]Dept. of Microbiology, Oklahoma State University)*
Pseudomonas aeruginosa was used as a model system to study stress-induced genetic alterations in bacterial cells.

Effects of Environmental Factors on Bacterial Conjugation, *M.A. Gealt, T.A. Khalil, and S. Selvaratnam, Dept. of Bioscience and Biotechnology, Drexel University*
The effect of different factors on transcription of tra and mob as might occur in wastewater were studied to elucidate potential genetic interactions between released bacteria and indigenous populations.

The Fate, Stability & Movement of Foreign DNA in Filamentous Fungi: An Environmental Study, *M.B. Dickman[1] and J.F. Leslie[2] ([1]Dept. of Plant Pathology, University, Dept. of Plant Pathology, [2]Kansas State University)*
The stability of foreign DNA sequences in fungi was evaluated to enhance the understanding of potential ecosystem effects of DNA introduced via an eukaryotic vector.

Modeling Transduction in Aquatic Environments, *R.V. Miller[1], T.A. Kokjohn[2], and G.S. Sayler[3] ([1]Dept. of Microbiology, Oklahoma State University, [2]Argonne National Laboratory, IL, [3]Center for Environmental Biotechnology, University of Tennessee)*
Pseudomonas aeruginosa is being used as a model to study viral-mediated gene transfer (transduction) in freshwater microbial populations.

Stability of Foreign DNA in the Fungus *Colletotrichum gloeosporioides*, *J.L. Armstrong[1] and D.L. Harris[2] ([1]U.S. EPA, Corvallis, OR, [2]ManTech Environmental Technology, Inc.)*

The fungus *C. gloeosporioides var. aeschynemone* (CGA) was selected as a model organism to study the factors that determine the stability of transformed marker genes under simulated environmental conditions.

Genomic Plasticity and Catabolic Potential of *Pseudomonas cepacia*, *T.G. Lessie, A. Ferrante, A. Byrne, M.S. Wood, and H.P. Cheng, Dept of Microbiology, University of Massachusetts*
The construction of a physical map of the chromosome of *P. cepacia* has been undertaken to provide insight into the basis for this bacterium's novel catabolic functions and extraordinary nutritional versatility.

Application of DNA Hybridization to Compare Bulk DNA's from Different Soils, *L.A. Porteous and J.L. Armstrong, U.S. EPA, Corvallis, OR*
A rapid, small-scale method of extracting DNA pure enough for use in hybridization experiments was developed. When coupled with dot-blot techniques, DNA extracts can be compared and quantified.

Update on the Assessment of Bacterial Dispersal From Foliage by Rain, *H.A. McCartney and J. Butterworth, A.F.R.C. Institute of Arable Crops Research, Rothamsted Experimental Station, Harpenden, Herts, England*
The bacterial content of run-off from leaves and of water droplets dispersed by splash were assessed.

Field Release of a Genetically-Altered Baculovirus with a Limited Survival Capacity, *H.A. Wood and P.R. Hughes, Boyce Thompson Institute, Ithaca, NY*
This project is being conducted to evaluate the spread and the survival potential of a genetically altered virus within the wild-type population. The release was conducted with an altered *Autographa californica* nuclear polyhedrosis virus (AcMNPV) which has a deleted polyhedron gene, but no foreign gene inserted.

Modeling the Fate of Bacteria in Surface Waters, *J.P. Connolly[1], R.B. Coffin[2], and R.E. Landeck[1] ([1]Environmental Engineering & Science Program, Manhattan College, [2]U.S. EPA, Gulf Breeze, FL)*
The overall objective of this project is the step-wise development of the components of a framework for modeling the movement and growth of bacteria that have been introduced into a surface water system.

Epiphytic Fitness Genes and Phenotypic Adaptation, *Steven E. Lindow, Dept. of Plant Pathology, University of California*
Studies have been initiated with *Pseudomonas syringae* to determine the range of differences in epiphytic fitness exhibited by a given genotype of bacterium that is exposed to different environments prior to inoculation onto plant surfaces, and to determine novel fitness determinants.

Molecular Studies of Microbial Ecosystem Perturbations, *D.A. Stahl, University of Illinois*
Techniques have been developed using comparative rRNA sequencing and hybridization for use in the characterization of microbial populations in natural communities, thus avoiding limitations associated with pure culture isolation.

Habitat Specific Differences in Persistence and Effects of Introduced Cellulolytic Bacteria Used as Surrogates for GEMs, *T. Bott and L.Kaplan, Stroud Water Research Center, Academy of Natural Sciences, Avondale, PA*
The primary objectives of this research were to determine the persistence of introduced

bacteria in natural benthic aquatic communities, to identify response variables most useful for detecting effects on introduced organisms, and to evaluate the utility of mesocosms for assessing the fate and effects of GEMs in natural systems.

Comparison of xylE Gene Activity in Different Molecular Constructs, *H.M. Abebe[1], R.J. King[1], S.E. Lindow[2], K.A. Short[3], and R.J. Seidler[3] ([1]ManTech Environmental Technology, Inc., [2]Dept. of Plant Pathology, University of California, [3]U.S. EPA, Gulf Breeze, FL)*
The stability and level of expression of plasmid-borne and chromosomally inserted xylE marker genes, and assay systems for these marked bacteria were assessed.

B. Environmental Effects Studies

Genetic and Molecular Analysis of Survival Mechanisms of Pseudomonads, *A.J. Anderson, R. Buell, J. Katsuwon, C. Heck, and R. Zdor, Biology Dept., Utah State University*
Basic processes involved in colonization of plant roots by pseudomonads were studied.

Route Specificity of the Toxicity of the *Bacillus thuringiensis subsp. israelensis* 28 Kilodalton Protein, *C.Y. Kawanishi and M.E. Mayes, U.S. EPA, Research Triangle Park, NC*
Studies were conducted to ascertain the effects of different routes of challenge with the solubilized Bti parasporal crystal 28 kDa polypeptide in rats. Changes in serum parameters after intraperitoneal challenge were monitored.

The Effect of a Genetically Altered Bacterium on Nitrogen Transformation Rates, Indigenous Microbiota, and Microbial Biomass in a Xeric Soil, *L.K. Gander[1], E.R. Ingham[2], J.D. Doyle[1], and C.W. Hendricks[3] ([1]ManTech Environmental Technology, Inc., [2]Dept. of Botany and Plant Pathology, Oregon State University, [3]U.S. EPA Environmental Research Laboratory, Corvallis, OR)*
This study examined the dynamics of indigenous populations of bacteria, protozoa, and nematodes in untreated soil, and in soil treated with 2,4-dichlorophenoxyacetate (2,4-D), and amended with a *Pseudomonas* strain engineered to degrade this herbicide.

Effect of Organochlorine Compounds on the Bioactivation of 2,6-Dinitrotoluene in Fisher-344 Rats, *S.E. George, R.W. Chadwick, M.J. Kohan, and J.C. Allison, U.S. EPA, Research Triangle Park, NC*
The effect of organochlorine compounds on metabolism and on GI tract flora were evaluated.

Clearance of Environmental Pseudomonads from CD-1 Mice Following Intranasal Exposure, *S.E. George, M.J. Kohan, D.A. Whitehouse, and L.D. Claxton, U.S. EPA, Research Triangle Park, NC*
The potential health effects associated with intranasal exposure to engineered organisms were assessed by monitoring of morbidity and mortality of the host, and survival of microorganisms in the lungs, nasal washing, and intestinal tract.

Fate of *Bacillus sphaericus* Microbial Pest Control Agent In the Environment, *A.A. Yousten and E.F. Benfield, Biology Dept. Virginia Polytechnic Institute and State University, Blacksburg*
This research examines the fate of bacterial spores during their interaction with the aquatic environment as well as the fate of

spores ingested by certain nontarget invertebrates.

In Vitro Survival and Competition of Environmental Psuedomonads Within a Human Fecal Flora Culture, *G.M. Nelson[1], L.D. Claxton[2], and S.E. George[2] ([1]Environmental Health Research and Testing, Inc., [2]U.S. EPA, Research Triangle Park, NC)*
This study is a continuation of research into the potential adverse health effects due to the environmental release of microorganisms.

Laboratory Observations of the Inhibition of Soil Fungi by MPCA's of the Genus *Pseudomonas*, *H.M. Abebe[1], V.P. Fieland[1], and R.J. Seidler[2] ([1]ManTech Environmental Technology, [2]U.S. EPA, Corvallis, OR)*
This work, an assessment of interactions between bacteria and soil fungi, is a continuation of a study which demonstrated reduction of soil fungal populations due to an accumulation of a metabolic intermediate (2,4-dichlorophenol) of 2,4-D degradation.

Interactions of Nontarget Invertebrates with *Bacillus thuringiensis* var *israelensis* in Natural Ponds, *R.L. Anderson[1], E. Mead[2], L.J. Shannon[3], and D. Janssen[3] ([1]U.S. EPA, Duluth, MN, [2]American Scientific International, [3]Biology Dept., University of Minnesota)*
The objective of this project was to determine, in temporary pools treated with control mosquitoes, whether or not nontarget animals accumulated and retained the spores of Bti.

Current Investigations on Microsporidian Test Systems, *W.S. Fisher, J.W. Fournie, C.L. McKenney, Jr., and D.P. Middaugh, U.S. EPA, Gulf Breeze, FL*
A totally enclosed aquarium has been used as a test system to assess the effect of a variety of microbial pest control agents (MPCA's) on marine and freshwater nontarget species. Current focus is on microsporidian (unicellular, obligate intracellular parasites) MPCA's.

Use of Ribosomal rRNA Sequences to Characterize Diversity and Stability of Microbial Populations, *R. Devereux, Technical Resources, Inc., Gulf Breeze, FL*
This research utilizes 16S rRNA-targeted hybridization probes to define populations of sulfate-reducing bacteria and assess population stability in relation to ecological processes.

An Overview of Protocol Development for Avian Pathogenicity Tests, *A. Fairbrother[1] and P. Buccholz[2] ([1]U.S. EPA, Corvallis, OR, [2]ManTech Environmental Technology, Inc.)*
This report is a review of the development of standardized protocols that can be used to determine the pathogenicity of microorganisms in nontarget avian species. Emphasis is placed on recent developments in respiratory protocols.

Test Procedures for Assessing Hazards of Microbial Pest Control Agents to Freshwater Fish, *Virginia M. Snarski, U.S. EPA, Duluth, MN*
The interactions between fish and the registered MPCA, *Bacillus thuringiensis* subsp. *israelensis* (Bti), under laboratory conditions, were investigated.

Field Calibration of Soil-Core Microcosms for Evaluating Fate and Effects of Genetically Engineered Microorganisms in Terrestrial Ecosystems, *H. Bolton, Jr. and J.K. Frederickson, Battelle Northwest Pacific Laboratory, Richland, WA*

This work focused on calibration of soil-core microcosms with their field counterparts for microbial fate and ecosystem structural and functional properties, and the resultant effect of introduced microorganisms on these properties.

Development of Test Methods to Assess Fate of Microbial Pest Control Agents and Their Effects on Nontarget Aquatic Organisms, *F.J. Genthner, G.M. Cripe, and D.P. Middaugh, U.S. EPA, Gulf Breeze, FL*
A fully contained single species test system was developed to determine whether exposure of a nontarget aquatic invertebrate to a microbial pest control agent (MPCA) will result in infectivity, toxicity, or pathogenicity.

Protocol Development for Testing the Effects of Bacterial Pesticides on Beneficial Beetles, *R. James[1] and B. Lighthart[2] ([1]Mantech Environmental Technology, Inc., [2]U.S. EPA, Corvallis, OR)*
In order to develop a standard assay for testing the effects of bacterial pesticides on beneficial insects, the effect of temperature, dietary stress, and larval instar on the susceptibility of *Hippodamia convergens* (the convergent lady beetle) to a weak bacterial pathogen was tested.

Use of 16S rRNA Probes to Correlate Sulfate Reducer Community Structure with Mercury Methylation, *M.R. Winfrey and J. Winfrey, Dept. of Biology and Microbiology, University of Wisconsin*
Sulfate reducing bacteria (SRB) are known to be active methylators of mercury, and were found to comprise a larger portion of the microbial population in low pH lakes in northern Wisconsin. The use of SRB-specific probes provides previously unattainable information on the community structure of these microorganisms which play a key role in nutrient cycling in anaerobic environments.

Assessing Host Specificity of Fungal MPCA's to the Beneficial Wasp Trichogramma pretiosm, *D.K. Sewell[1] and B. Lighthart[2] ([1]ManTech Environmental Technology, [2]U.S. EPA, Corvallis, OR)*
This study was undertaken to develop bioassay procedures to evaluate pathogenicity of entomogenous fungi, compare specific and nonspecific fungi, and evaluate factors that may affect the expression of fungal virulence to *T. pretiosum* or the susceptibility of *T. pretiosum* to fungal pathogens.

Field Validation of Laboratory Microcosms Using *Bacillus thuringiensis* var. *israelensis*, *L.J. Shannon, D.M. Janssen, and R.L. Anderson*
Both mixed flask culture and core microcosms were evaluated to determine whether they accurately and sensitively reflect Bti survival, effects of Bti on target and nontarget species, and influence of physical, chemical, and biological conditions in natural systems on monitoring procedures.

Effects of a Lignin-Degrading Recombinant Streptomyces on Microbial Activity and Nutrient Cycling in Soil, *D.L. Crawford[1], Z. Wang[1], J.D. Doyle[2], H. Bolton, Jr.[3], J.K. Fredericksen[3], S.A. Bentjen[3], and C.W. Hendricks[4] ([1]Dept. of Bacteriology and Biochemistry, University of Idaho, Moscow, [2]ManTech Environmental Technology, Inc., [3]Battele Pacific Northwest Laboratory, Richland, WA, [4]Environmental Research Laboratory, Corvallis, OR)*
The effects of release of recombinant *S. lividans* on bacterial and fungal activity, and on carbon and nitrogen cycling in soil were evaluated.

Mechanisms of Effects of Recombinant Streptomyces on the Carbon Cycle in Soil, *D.L. Crawford, Z. Wang, and B.H. Bleakley, Dept. of Bacteriology and Biochemistry, University of Idaho, Moscow*
The genetic and chemical bases for the effects of recombinant S. lividans strains expressing lignin peroxidase on carbon cycling were examined.

C. Control Strategies

Comparison of Survival on the Phylloplane of Bacteria Released in Greenhouse and Field Experiments, *K. Donegan[1], J. Armstrong[2], C. Matyac[1], and R. Seidler[2] ([1]ManTech Environmental Technology, Inc., [2]U.S. EPA, Corvallis, OR)*
The use of greenhouses for predicting the results of field releases, and the influence of bacterial species, plant species, and environmental conditions were investigated.

Potpourri of Bioaerosol Research at ERL-Corvallis in 1990, *B. Lighthart[1], B.T. Shaffer[2], and B. Marthi[2] ([1]U.S. EPA, Corvallis, OR, [2]ManTech Environmental Technology, Inc.)*
This report summarizes progress in 1990 in (1) increasing bioaerosol sampling efficiency, (2) development of better tools (Auto-DAT and EBARC) to evaluate bioaerosol generation potential, and (3) development of predictive simulation models of bioaerosol dispersal and deposition.

Measuring Entrainment of Bacteria From the Phyllosphere, *M. Walter[1], V. Fieland[1], L. Ganio[1], and R. Seidler[2] ([1]ManTech Environmental Technology, Inc., [2]U.S. EPA, Corvallis, OR)*
A method of artificially inducing bacterial entrainment from the phyllosphere was developed, and various sampling methods for detection of entrained bacteria were compared. The effect of species of host plant and the effect of entrainment on bacterial viability were assessed.

Environmentally Released Microorganisms and the Enteric Microflora, *W. Dobrogosz[1], Y. Lin[1], M. Fiuzat[1], E. George[2], and L. Claston[2] ([1]Dept. of Microbiology, North Carolina State University, [2]U.S. EPA, Research Triangle Park, NC)*
Methodologies for growing enteric anaerobes were compared, and various bacterial species from mouse intestines were enumerated.

Biotechnology Engineering Risk Management, *J. Burckle, U.S. EPA Risk Reduction Engineering Laboratory*
A number of reports have been written addressing concerns associated with large-scale production of biotechnology products. These concerns include worker exposure, release of GEMs from production sites, and the efficacy of containment and destruction techniques.

Biotechnology Quality Assurance, *A. Smiecinski and L. Stetzenbach, Environmental Research Center, University of Nevada, Las Vegas*
A document intended to assist scientists in understanding and implementing QA principles as they apply to biotechnological projects has been produced.

The Use of Lethal Bacterial Genes to Limit the Survival of Intentionally Released Genetically Engineered Microorganisms, *W.H. Jeffrey[1], S.M. Cuskey[2], and R.B. Coffin[2] ([1]Technical Resources, Inc., [2]U.S. EPA, Gulf Breeze, FL)*
Effects on growth, lethality, and mechanism of action of the RK2 gene kilA were examined.