Appendixes

Appendix A

Pending and Potential Planned Introductions of Genetically Engineered Organisms

The information contained in this appendix is grouped according to the organism receiving the transferred genetic material, or, in the case of deletions, the organism from which genetic material is deleted. Within any given entry, pending applications (those at or near the field test stage) are described first. In some cases, material on likely or possible applications follows. This information was compiled from publicly available materials, and was last updated in early 1988.

Microbes

Bacteria

Killed Bacteria as pesticide (Mycogen, San Diego, CA).—Mycogen scientists cloned the delta-endotoxin gene from ***Bacillus thuringiensis*** (BT) and inserted it into a strain of ***Pseudomonas*** via plasmid-mediated transformation. The ***Pseudomonas*** is cultured to produce large amounts of the delta-endotoxin, a protein that is toxic to lepidoptera larvae. The bacteria are then killed, their cell walls fixed, and the resulting 'poison capsule' is administered as a topical insecticide. Using the killed and fixed bacteria as a delivery vehicle increases the longevity of the toxin in the environment. In other applications the protein is rapidly degraded by sunlight and other environmental action. Using killed bacteria also eliminates most containment problems. Small-scale field trials were conducted in test plots near Orlando, FL.

Toa Gosei Chemical, in Japan, has an active research program aimed at producing similar killed BT agents. They are screening natural strains of B. ***thuringiensis*** to identify varieties that will target specific pests (e.g., diamond back moth, fabricius, etc.) and cloning the toxin genes into ***Escherichia coli*** and B. ***subtilis*** to enhance production (37).

"Ice Minus" (Advanced Genetic Sciences (AGS), Oakland, CA).–AGS researchers have produced strains of ***Pseudomonas syringae*** and ***P. fluorescent*** from which the gene for the ice-nucleation protein has been deleted. AGS had hoped to field test in early 1986, but legal challenges, local opposition, and controversy over unauthorized facilities for pathogenicity tests mandated by the Environmental Protection Agency (EPA) caused delay. The first field test began on April 24, 1987, near Brentwood, in Contra Costa County, CA.

The bacteria were topically applied to 2,400 strawberry plants to test the degree of frost resistance conferred upon strawberry plants in the 0.2-acre plot, as well as to monitor such risk assessment parameters as survival and dispersal. The manipulations involved do not impart any new genetic information; rather, they delete existing information. Comparable strains of bacteria occur naturally, the results of random mutation. Potential for ultimate survival or spread of test strains has been judged low. There is no likelihood of novel characters being transmitted to nontarget species. EPA announced approval of the application for an experimental use permit in February 1987.

A similar experiment was first proposed in 1982 by Steven Lindow and Nickolas Panopoulos of the University of California at Berkeley to delete the gene for the ice-nucleation protein from strains of ***P. syringae and Erwinia herbicola*** and field test them for increased frost resistance of host plant substrates (especially potato plants). EPA and the Recombinant DNA Advisory Committee of the National Institutes of Health both approved the proposal. Local opposition and legal challenges by the Foundation on Economic Trends made a Fall 1986 test impossible. The field test commenced on 29 April 1987, near Tulelake, CA, with the planting in a half-acre plot of 2,000 inoculated tubers and 2,000 controls.

"Ice plus" (Snomax Technologies, Oakland, CA).–Naturally occurring strains of spray-dried ***P. syringae*** (produced by Kodak Bioproducts Division for AGS), containing high concentrations of the ice-nucleation protein, are mixed with water and sprayed from snowmaking guns at ski resorts. This procedure is from 20 to 80 percent more efficient than water alone, and has been tested on ski slopes in Colorado, Michigan, Minnesota, New York, and Vermont. The 1986-87 season saw large-scale use in a number of resorts (a major delay until recently has been in production facility availability and capacity. Use has been approved 011 U.S. Forest Service lands. It is also possible that the bacterium might be used in a variety of ice-making applications in the Arctic.

AGS claims they have also developed a recombinant-DNA form of the bacterium that is up to 1,000 times as effective as the wild-type strain. There are no immediate plans to use this in any environmental applications.

Engineered Microbial Pesticide (Monsanto, St. Louis, MO).—Monsanto scientists have cloned the delta-endotoxin gene from B. ***thuringiensis*** and used transposable elements to install it in the chromosomes of strains of ***P. fluorescent,*** **a** microbe that colonizes the surfaces of corn plant roots. In its host, the inserted gene is expressed and the gene product retains its toxicity. Monsanto plans to inoculate seed corn with the engineered bacteria in the hope that they will colonize the roots of the developing plant. The insecticidal activity of the protein toxin should then protect the plant from the corn-root cutworm. Monsanto's proposed field test would involve planting 27,000 coated seeds on a l-acre test plot for each of two consecutive years.

While data on the behavior of transposable elements in other systems (notably *Drosophila)* suggest caution is warranted, potential problems associated with horizontal gene transmission seem to have been preempted in this case. Monsanto scientists inserted the delta-endotoxin gene into the ***Pseudomonas*** chromosome with a disarmed transposable element-one from which the transposase gene (necessary for element mobility) was deleted. Insertion was affected with the aid of a "helper" transposable element, from which one of the long terminal repeats necessary for insertion was deleted (25,26,27).

Monsanto has submitted to EPA a proposal for an Experimental Use Permit for field testing. EPA has asked for data from additional toxicity protocols, which are now being performed.

Toxic Waste Disposal.-Bacteria maybe engineered to enhance or receive capabilities to metabolize or sequester specific toxic wastes, including polychlorinated biphenyls (PCBs), polybrominated biphenyls (PBBs), dioxin, oil spills, phenol, pesticides, herbicides, and heavy metals. A host of bacteria capable of metabolizing different substrates are known (17). One such bacterium is a strain of ***Pseudomonas*** (developed by Ananda Chakrabarty) that has been given the capability (Plasmid-qmediated) to metabolize several components of crude oil. This may enhance the possibilities to control oil spills biologically. It differs from established techniques by combining several different carbon degradative pathways into one organism. Existing methods generally use a mixture of naturally occurring bacteria each with the capacity to metabolize a different crude ail component, EPA researchers in Gulf Breeze, FL, have developed systems capable of degrading jet fuel, 2)4,5-T (the active ingredient in Agent Orange), trichloroethylene (a solvent that is the most common ground water contaminant), and pentachlorophenol (PCP, a common wood-treatment chemical).

Many naturally occurring bacteria have the ability to degrade a variety of chemicals found in toxic waste. These abilities could be enhanced both by classical selection and genetic engineering. Species of ***Achromobacter*** are able to degrade carbofuran, a pesticide against corn rootworm and other crop pests. ***Flavobacterium*** species are known to degrade coumaphos, a pesticide used against livestock pests. An atrazine resistance gene has been isolated from cyanobacteria, or blue-green algae. Such bacteria maybe engineered for environmental cleanup programs, such as degrading toxic material remaining in storage tanks where farmers dump pesticide or herbicide wastes,

The EPA lab at Gulf Breeze has developed a "suicide" plasmid to ensure that a gene encoding a 3-chlorobenzoic acid-degrading enzyme will be destroyed when it has served its purpose. The plasmid contains four elements: the gene for the degradative enzyme, a promoter, a methylase gene, and a restriction enzyme-encoding gene. In the presence of 3-chlorobenzoic acid, the promoter induces production of both the degrada - tive enzyme and the methylase, which protects the degradative gene from the action of the restriction enzyme. When the acid has been completely degraded, methylase production is suppressed, and the restriction enzyme digests the degradative gene. Scientists believe this type of scheme should be applicable to many other metabolic pathways, though there are no imminent plans to field test such microbes.

Toxic Waste Disposal or Heavy Metal Recovery (Mining). -Bacteria might be engineered to enhance their abilities to extract or concentrate heavy metal contaminants from land fills or mine tailings. The bacteria would promote the efficient recovery of minerals, minimize existing pollution, and leave reclaimed land usable for agriculture. A number of existing mining operations use bacteria to aid in the recovery process, such as cobalt extraction with ***Thiobacillus ferrooxidans.*** Other potential targets include lead, cadmium, and copper,

Researchers at the EPA laboratory in Gulf Breeze, FL, are developing bacterial strains resistant to mercury, with the intention of using them to reduce pollution problems in mercury contaminated environments.

It may also be possible to alter some species of algae to enhance or impart toxic waste disposal ability (see section on Plants: Engineered Algae),

Pollution Control.-Phosphorus removal, ammonia oxidation, and flocculation are three significant problems facing municipal water purification systems. Bacteria could be engineered to aid each of these tasks.

Miscellaneous—Ecogen (Princeton, NJ) is using both classical and biotechnological approaches to develop a variety of novel pesticides. "Plasmid curing" has produced some new BT toxin vehicles, and recombinant DNA techniques are being used to investigate potential pesticide uses of B. ***subtilis*** toxin genes.

After normal drilling operations, as much as 50 percent of the oil may remain in wells, due to capillary forces and chemical adhesion (8). Engineered bacteria may help extract that remaining oil.

Microbes may be tapped to aid in biomass energy production, as with yeast engineered to enhance their ability (generally in contained facilities) to produce ethanol for fuel. Scientists at the University of Florida and elsewhere are currently studying this possibility.

Bacteria may also be engineered to enhance their function in food production processes (28).

Viruses

Viruses as Pesticides.—By manipulating the organization and expression of baculovirus genomes, researchers hope to increase baculovirus utility as pesticides with specific applications. Commenting on potential risks, one researcher has said '(My guess is no problem but there are a variety of constructs possible, and some could have broader effects than desired. . . . One can consider many scenarios. In the worst case (also the most improbable) the situation could not be corrected. . . . In the course of our studies already we have developed what we consider improved methods of assessing risks of genetically engineering viral pesticide products. . . . Our methods of assessing viral gene expression in nontarget hosts are extremely sensitive" (19). work is being done in this area by at least three academic groups (in Florida, Texas, and Georgia) and two commercial companies (Genetics Institute, Cambridge, MA, and MicroGeneSys, West Haven, CT).

Researchers at the Institute of Virology in Oxford, England, are pursuing several similar applications. The first involves a baculovirus that is pathogenic to the pine beauty moth, ***Panolis flammea,*** a pest of lodgepole pine (and other species), especially in northern Scotland. Field tested viruses contain special marker nucleotide sequences, to aid in tracking distribution and dispersal. Although approval has been obtained from the appropriate regulatory bodies (the Advisory Committee on Genetic Manipulation of the Health and Safety Executive, and the Forestry Commission) as well as the Nature Conservancy Council, delays made testing impossible in 1986. Field tests took place during 1987. (British regulation is largely voluntary, and somewhat more flexible than at present in the United States.)

A similar test to measure viral persistence in a given ecosystem involved a baculovirus ***(Autographa californica*** nuclear polyhedrosis virus) pathogenic to caterpillars of the mottled willow moth, ***Spodoptera exigua.*** This test was carried out by Institute of Virology scientists at an undisclosed United Kingdom site during the summer of 1986 (3), In both cases, the scientists anticipate inserting genes to increase the virulence of the viral pathogen. The gene for the BT delta-endotoxin is a likely candidate.

Genetics Institute (Cambridge, MA) is pursuing research aimed at inserting an insect toxin gene into a nuclear polyhedrosis virus. The engineered virus is targeted at *A.* ***californica,*** an alfalfa pest, as well as Heliothis species, pests of various crop plants including cotton, soybeans, and rice. Similar work on related viruses is under way at Tottori University, in Japan.

Commonwealth Scientific and Industrial Research Organization (CSIRO) researchers in Australia are exploring genetic engineering techniques as a means of restoring the virulence of the myxoma virus. Originally introduced to control pest populations of introduced rabbits, attenuated forms of the initially virulent virus evolved at the same time rabbits developed increased resistance. Although the original virus devasted rabbit populations, the subsequent coevolution between host and pathogen has led rabbit numbers to once again increase. Scientists are attempting to add a bacterial toxin to the viral genome to increase its potency (42).

Viruses in Engineered Vaccines.--On 23 July 1986, the Food and Drug Administration (FDA) announced approval of "Recombivax HB", " a genetically engineered vaccine for hepatitis B, a disease carried by as many as 200 million people worldwide, 700,000 in the United States alone. Based on the hepatitis B cell surface antigen, the vaccine was developed by Chiron ***(Emeryville, CA) and marketed by*** Merck & Co. It has been shown to produce the same high level of immunity in clinical trials as Merck's blood-plasma-based vaccine, but without the potential worries associated with contamination of donated blood by the AIDS (acquired immunodeficiency syndrome) virus. Several Japanese groups are working on an engineered hepatitis B vaccine, as are researchers at the Pasteur Institute, Paris; at Merck, Sharp, & Dohme in West Germany; and at Smith Kline Biological, Belgium (29).

A cooperative group at the Japan Polio Research Institute and the University of Tokyo Medical School has recently announced the construction of an attenuated polio virus vaccine that retains high immunogenicity. Additional competitors are emerging (40). Japanese research is also focussed on engineered herpes simplex viruses as vaccines against several human diseases.

Chiron is also working on the development of vaccines against hepatitis A, AIDS, malaria, and oral and genital herpes. In October 1986, Chiron announced a joint venture (the Biocine Company) with Ciba-Geigy to produce genetically engineered vaccines.

Researchers at the National Institute of Allergy and Infectious Disease (NIAID) and elsewhere have inserted into the vaccinia virus (used originally for smallpox vaccinations) genes responsible for antigen production in a number of diseases—herpes simplex, influenza, hepatitis B, rabies, and respiratory syncitial virus, all in humans; and vesicular stomatitis in cattle, horses, and pigs. Vaccinia virus is particularly valuable as a vector because it needs no refrigeration; it is cheap, easy to administer, has a large capacity for foreign DNA; and it has proved safe and effective in over 200 years of use. An early case of inadvertent release involved the accidental vaccination of laboratory researchers during a test in mice of a vaccine for vesicular stomatitis (13).

A vaccine for malaria is being pursued by several research programs, including groups at New York University, Chiron, NIAID (working with vaccinia systems), and a collaborative effort by Smith, Kline & French and the Walter Reed Army Institute of Research. Preliminary human trials began on 17 March 1986, with a vaccine developed by the latter. Early reports of results were disappointing.

Peter J. Hotez and colleagues at Rockefeller University are developing a vaccine against hookworm, two species of which (Necator americanus and *Ancyclostoma duodenale)* cause a substantial public health problem worldwide. The antigenic determinant for the vaccine is the histolytic proteolytic enzyme (HP), which attaches the hookworm to the wall of the small intestine. Synthetic antigen in the vaccine stimulates the host to produce antibodies to the enzyme. Researchers used phage lambda gt11 to clone the gene for HP necessary for large-scale production.

Australian scientists at CSIRO, working with Biotechnology Australia and Arthur Webster, two Australian biotechnology companies, have developed a vaccine for sheep foot rot, caused by ***Bacteroides nodosus.*** The gene from this bacterium, encoding the production of the cell surface filaments crucial to infection, was transferred to P. ***aeruginosa.*** Inoculating sheep with the altered P. *aeruginosa* bacterium stimulates the desired immune response.

Different groups in the European Economic Community are pursuing engineered vaccines for the chicken pathogen, infectious bronchitis virus (IBV), foot and mouth disease, bovine and swine rotavirus, bovine leukemia, avian erythroblastosis, swine pseudora - bies, and rabies.

Scientists at Baylor University and at Texas A&M field tested a vaccine for swine pseudorabies in 1984. An investigation by the National Institutes of Health's (NIH) Recombinant DNA Advisory Committee found the researchers remiss for not having consulted the local Institutional Biosafety Committees, and concluded that ambiguities in the NIH guidelines contributed to confusion in the case. The vaccine was developed by Novagene, Inc. (Houston) and is now commercially available through TechAmerica Group, Inc. (Omaha, NB).

In 1986, researchers from Oregon State University, at the invitation of collaborators in New Zealand, field tested an engineered vaccinia virus in that country. The research was designed to study immunogenicity, pathogenicity, and transmissibility of a model vaccinia virus in sheep, calves, and chickens. The engineered vaccine contained structural genes for proteins from sindbis virus, a single-stranded RNA virus (31). The researchers found the tests successful.

Scientists from the Wistar Institute (Philadelphia) developed, and collaborators from the Pan American Health Organization field tested, an engineered vaccinia virus vaccine against rabies in Argentina in 1986 (see ch. 3).

Vaccines (especially those using vaccinia virus) may be engineered to carry simultaneously the antigenic determinants for a series of diseases, related or not (20,30). These vaccines must be monitored carefully, to avoid the possibility of recombination producing newly virulent viral forms (12).

In the face of antigenically complex systems, such as malaria or sleeping sickness, engineered multivalent vaccines may be the only way to provide general protection. Work is under way to produce such engineered vaccines (4 I), but estimates place successful completion 10 or more years in the future. However, preliminary successes with previously refractory diseases, such as schistosomiasis (27), may mean that single antigen approaches might be fruitful against systems once thought amenable only to multivalent vaccines.

Plants

Herbicide Resistance

Glyphosate.—Scientists at Calgene (Davis, CA) have inserted the ***aroA*** gene from ***Sahnonella typhimurium*** into tobacco to confer tolerance to the herbicide glyphosate (e.g., Monsanto's Roundup"). A disarmed plasmid from ***Agrobacterium rhizogenes*** served as the vector (7). While this gene transfer imparts a new characteristic to strains of an existing plant species, the

chance that these characteristics might spread seem to be very low, if not zero. The U.S. Department of Agriculture (USDA) approved Calgene's applications to field test the altered tobacco plants.

Calgene has patented the ***aroA*** gene under the name GlyphoTol and plans to file applications soon to test similarly transformed tomato and cotton plants. Corn, soybean, rape, and some trees are targets of similar efforts. Calgene has also been working on transforming oil rape ***(Brassica napus)*** with a bacterial antibiotic resistance gene.

Researchers at Monsanto have used the Ti plasmid from ***A. tumefaciens*** to insert genes for glyphosate resistance into tobacco, tomato, and petunia cells from which resistant plants have been regenerated (34). Field tests were conducted in the summer of 1987.

Atrazine (Ciba-Geigy).-The herbicide atrazine, manufactured primarily by Ciba-Geigy and marketed as AAtrex, is degraded very slowly in the environment. An effective herbicide, annual sales of AAtrex amount to nearly $250 million, making it the second best selling herbicide after Roundup@. Atrazine is commonly used on corn, which is naturally resistant. But because it persists in soil, it can contaminate runoff and reduce yields in sensitive crops that are planted in rotation with atrazine-treated corn, a problem termed "carry-over." Several crops are likely candidates for the induction of atrazine resistance. According to one estimate, atrazine resistance in the prominent varieties of soybeans would allow sales of the herbicide to double or triple (2 I). Field tests of engineered atrazine resistance in tobacco were carried out by Ciba-Geigy in North Carolina in 1986.

The gene for atrazine resistance was transferred by cross-breeding from bird's rape *(Brassica campestris)* to oil rape, or canola (B. ***napus),*** which was field tested on 30,000 acres in Canada in 1984.

Sulfonylurea (Du Pont).-Genes for resistance or tolerance to the sulfonylureas, such as chlorsulfuron (Glean, used on grains, especially wheat and barley) and Oust @ (a broad spectrum herbicide), are the targets of research aimed at producing transgenic plants. Soil residues of these herbicides can damage crops grown in rotation, such as sunflowers or soybeans. Engineering resistance into rotation crops would protect them and expand the market for these herbicides. Work on this possibility has so far involved the production of plants transformed with resistance genes introduced into tobacco cells produced in tissue culture. Du Pont, collaborating with Northrup King, field tested resistant tobacco in 1987.

Miscellaneous.—Researchers at Molecular Genetics (Minnetonka, MN) are pursuing the induction of resistance to imidazolinone (made by American Cyanamid) in corn. Approaches based on cell culture and selective breeding are closest to commercialization.

Calgene (Davis, CA) scientists have isolated a gene conferring tolerance to bromoxynil, a herbicide made by Rhone-Poulenc Agrochimie, France. Several strategies are being explored to introduce the gene into sunflower plant cells and other plant tissues. USDA has approved field tests scheduled for 1988 of tobacco plants engineered to be tolerant of this herbicide.

In January 1987, scientists at Plant Genetic Systems, Belgium, reported the genetic engineering of tomatoes, potatoes, and tobacco plants "totally resistant" to the broad spectrum herbicide "Basta@, " produced by Hoechst AG, Frankfurt am Main, West Germany. The active ingredient in the herbicide, phosphinotricin, inhibits the plant enzyme glutamine synthetase. Phosphinotricin can be inactivated by acetylation. The gene for an acetylating enzyme has been isolated from a strain of streptomyces and inserted into target plants, protecting them from doses of Basta as high as 10 times those used normally for weed killing (23).

Disease Resistance

Crown Gall Resistant Tobacco (Agracetus, Middleton, WI).—Agracetus scientists used a disarmed Ti plasmid to transform tobacco with a gene conferring resistance to crown gall disease. NIH approved the company's application for small-scale field testing in 1986, and USDA's Animal and Plant Health Inspection Service (APHIS) reviewed the same proposal and certified it as involving "no plant pest risk. " Field testing began on 30 May 1986, in Middletown, WI. Agracetus is pursuing similar ends in corn and soybeans.

Agracetus scientists have also transformed cotton plants (Gossypium hirsutum) with an antibiotic resistance gene coding for neomycin phosphotransferase (38).

Engineered Viral Cross Protection (Monsanto/Washington University St. Louis, MO).—Researchers at Washington University, in collaboration With Monsanto, have engineered cross protection against TMV in tobacco plants. Resistance to TMV infection was conferred by inserting the TMV coat protein gene into the tobacco plant genome via the Ti plasmid from A. ***tumefaciens*** (l). Similar results have been achieved with tomato mosaic virus and alfalfa mosaic virus, suggesting that many plants can be engineered to produce viral disease resistance. A field test of tomatoes engineered to be resistant to tobacco mosaic virus was approved by USDA and begun on a one-third-acre plot near Jerseville, IL, on 2 June 1987.

Miscellaneous (Rothamstead Experimental Station, Harpenden, United Kingdom) .-Researchers have used

cell fusion to produce a potato variety resistant to the potato leaf roll virus. The parental strains are a domestic potato and a wild, South American potato. Britain's Advisory Committee on Genetic Manipulation approved a field test for the summer of 1987. Also approved were field tests to be carried out at the Plant Breeding Institute (Cambridge) of potatoes carrying two added bacterial enzymes, as models for possible future improvement efforts.

Pest Resistance

BT Toxin Protected Crops. -In 1985, the Belgian company Plant Genetic Systems acheived the first introduction of the delta-endotoxin gene from B. ***thuringiensis*** into a plant.

Rohm & Haas, collaborating with Plant Genetic Systems, has since used the Ti plasmid to insert the delta-endotoxin gene into tobacco plants, providing protection primarily against the tobacco hornworm, ***Manduca sexta.*** Field testing was successfully completed in 1986 in Dade County, FL, and Bolivar County, MS. Hybrid seed is expected to reach the market in the 1990s.

Monsanto researchers have recently used the Ti plasmid from ***A. tumefaciens*** to insert the BT toxin gene into tomato plants, to provide protection against lepidopterous pests. Field tests were carried out in the summer of 1987 (11).

Miscellaneous.—Plant geneticists in England (a collaboration between Agricultural Genetics Company and the Plant Breeding Institute, both in Cambridge) have used ***A. tumefaciens*** vectors to insert into tobacco plants a gene coding for a protein that is a natural inhibitor of insect trypsin, a digestive enzyme. The spectrum of insect resistance thus conferred is much broader than that of BT-toxin-based applications (24). Seeds might be engineered to produce or increase their natural production of other "antifeedants," such as canavanine, thus reducing losses of stored seeds or grain to insect pests.

Other researchers at the Plant Breeding Institute have begun 1987-88 field tests of potatoes into which a kanamycin resistance gene has been inserted, with an ***A. tumefaciens-derived*** vector, to aid in risk assessment and agronomic studies of transgenic forms of a well established cultivar.

Tolerance to Environmental Factors

Plants can be engineered to increase their tolerances to such limiting environmental factors as salinity, drought, or sensitivity to heavy metal toxicity. This artificial expansion of ecological niches could be exploited to bring marginal lands into agricultural use or to decrease problems of deforestation and erosion due to overexploitation (in the Sonora, Great Basin, Negev, and Sahel, for example).

Nitrogen Fixation Enhancements

The promise of using genetic engineering techniques to enhance the nitrogen fixation in some plants, and to bestow it on others, has been highly publicized, Symbiotic bacteria in nodules on the roots of nitrogen-fixing plants extract gaseous nitrogen from the atmosphere and convert it to chemical forms accessible to plants. The biochemical pathways in bacteria that perform this function usually involve **16 or 17 structural genes** and their associated regulatory sequences, usually referred to as the ***nif*** (nitrogen fixing) genes.

BioTechnica International (Cambridge, MA) has produced two potentially commercial strains of ***Rhizobium meliloti,*** a bacterium that forms nodular colonies on the roots of alfalfa plants. The strains have been engineered to enhance their nitrogen-fixing ability through the insertion, via disarmed plasmid vectors, of additional copies of their own regulatory genes. The company hopes that 1988 field tests will demonstrate a 15- to 20-percent increase in nitrogen-fixing ability.

British scientists at the Rothamstead Experimental Station have inserted a marker sequence into a strain of ***Rhizobium*** for a summer 1987 field test to monitor the extent of gene transfer between rhizobial strains in soil.

Much of the research aimed at imparting nitrogen-fixing ability to plants that do not have it naturally is focused on transferring the ***nif*** genes into the plant. Formidable technical problems are involved in transferring so much genetic material and ensuring its proper expression. Substantial progress with this more generally applicable approach may be 5 to 10 years away.

Engineered Algae

Donald Cheney and colleagues (Northeastern University, Boston, MA) are using protoplasm fusion techniques to tailor marine algae (especially *Dunaliel/a salina)* to increase the efficiency of production of beta-carotene, agar, and other algal byproducts, and George Melville (Australian National University, Canberra, and Westfarmers Algal Biotechnology Pty., Ltd., Perth) are using recombinant DNA techniques to achieve the same goal.

Michael T. Henzl and Benjamin Greene, at New Mexico State University, have described the ability of the common alga, ***Chlorella vulgaris,*** to sequester gold. Similar abilities to sequester other heavy metals are known in other alga (36). Genetic manipulation may one day be able to enhance such abilities.

Miscellaneous

Researchers at Michigan Technological University are exploring the use of *Agrobacterium* vectors to impart new qualities to larch trees. The objective is to induce disease and herbicide resistance, making this rapid growing, genetically malleable conifer more valuable for reforestation programs.

Fungi are being explored, both by classical methods (David Sands, Montana Sate University) and cell fusion techniques (Gary Harman, Cornell) as herbicides targeted against Canada thistle and spotted knapweed and as antidisease agents, respectively. In the latter case, cell fusion methods were used to produce hybrid strains of the soil fungus ***Trichocderma harzianum*** that will be applied via inoculation on the seeds of peas and cucumbers. Field tests at the New York State Agricultural Experiment Station Vegetable Research Farm near Geneva, NY, will test the ability of the inoculated strains to protect against diseases such as damping off and root rot, caused by other fungi. The Environmental Protection Agency approved the field tests on 8 September 1986.

Japanese scientists have used cell fusion techniques to produce a hybrid between red and Chinese cabbage, called "Bio-Hakuran. " The new plant displays many characteristics that are intermediate, but it contains a full chromosomal complement from each parent—18 and 20, respectively, for a total of 38. Researchers are developing the hybrid cabbage as a new truck crop.

Genetic engineering may be able to improve the nutritional value of some plants by increasing their content of seed storage proteins and other components—for example, lysine in corn. Engineering may increase forage crop efficiency by enhancing digestibility. Plants might also be engineered to function as producers of pharmaceuticals or specialty chemicals, such as particular oils or storage lipids (14). Gene engineering of oil-seed crops for quality and quantity of oils is being done by USDA, Sungene, Calgene, Unilever, and BioTechnica, Canada.

Animals

Fish

Heat-Shocked Salmon.–Supported partly by the American Tackle Manufacturers Association, fisheries researchers in Washington and Michigan are using heat shock to induce triploidy in developing salmon embryos (Coho ***(Oncorhynchus kisutch),*** and king, or Chinook ***(Oncorhynchus tshawytscha)).*** The chromosomal abnormalities induced by heat shock disrupt normal reproductive cycles, including the spawning runs that lead to death. Plans are to stock Lake Michigan with triploid fingerlings in the expectation that more trophy fish will result. Triploid fish, with their disrupted reproductive cycles, contain no new genetic material, and cannot produce offspring. One researcher has suggested that future stocking programs should use such triploids to eliminate reproductive competition with and potentially negative impacts on the gene pools of wild salmon (16).

Triploid grass carp, also produced by heat shock, are being studied for use as aquatic weed control agents in southern riverways and irrigation systems, especially in California and Florida (39).

Miscellaneous. -Other work on salmon aims at enhancing growth hormone production, either by introducing foreign structural genes or enhancing the function of existing regulatory genes. Work with striped bass and trout is attempting to increase their cold tolerance by inserting a gene derived from winter flounder. One researcher has stated that within 5 years it should be possible "to routinely introduce genetic traits into cultured and wild fish species" (35). A number of different laboratories, most outside the United States, are pursuing work of this sort (15).

Livestock

The technology exists to genetically alter farm animals to improve reproductive performance, weight gain, disease resistance, or coat characteristics (USDA, Beltsville; University of Washington; University of Pennsylvania; CSIRO, Australia)(5). Fertilized embryos from rabbits, sheep, goats, cattle, and pigs have already been successfully transformed with human growth hormone genes (10,18). Scientists in Australia are working on moving sheep growth hormone genes between different varieties of sheep (9).

A significant body of research is being directed towards engineering livestock animals to function as new pharmaceutical sources (6), although aspects of the isolation and purification of such products remain to be worked out **(4).**

Poultry

Researchers in the Poultry Research Laboratory, in East Lansing, MI, have succeeded in transforming developing chick embryos with the avian leukosis virus and with chick syncitial reticuloendotheliosis virus, both common disease-causing organisms in poultry (32,33). The demonstration of the retroviral vector derived from avian leukosis virus may make it possible to inoculate chickens against the virus, as well as to insert other genes of interest, such as those regulating growth or egg production rates or conferring resistance to other diseases.

Miscellaneous

Genetic engineering techniques hold promise for altering insect pests to serve as tools in pest control or eradication. Sterile male blowflies can be produced by mutagenesis and selection, These altered flies, expected to help eradicate the pests, were field tested over 1985-86 on Flinders Island, Australia, by a group of Australian scientists (22). Efforts to control tephritid pests with engineered medflies are under way at the University of Hawaii.

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