Symposium—Biotechnology at Work

BIODEGRADATION OF SELECTED PESTICIDES UNDER ANAEROBIC CONDITIONS. Duane F. Berry, Ji-Dong Gu*, Ronald H. Taraban*, Hubert L. Walker, Jr.*, and D. C. Martens*, Dep. of Crop and Soil Environmental Sciences, VPI&SU, Blacksburg, VA. 24061. Many intentionally applied herbicides eventually end up in non-targeted areas such as wetlands, sediments, and groundwater where anaerobic conditions often prevail. We evaluated the biodegradability of atrazine, cyanazine, and dicamba in wetland soils under nitrate reducing and methanogenic conditions. Wetland soil samples from two different wetland areas located near the Chesapeake Bay were used to set up air tight serum bottle microcosms, containing soil, mineral-salts medium, and herbicide. Samples were withdrawn periodically by syringe to determine concentrations of methane (headspace), nitrate, and herbicide. Dicamba was degraded in both soils within 60 days under methanogenic conditions and in only one of the soils under nitrate reducing conditions. Since sterile controls for 2 of the 4 situations tested failed, transfer cultures were initiated in an effort to substantiate the biodegradability of dicamba. Decreases in concentration of atrazine and cyanazine occurred over an 8-month incubation time period in both soils under either nitrate reducing or methanogenic conditions (autoclaved sterile controls showed concentration decreases). Our results provide strong evidence that dicamba is biodegraded in wetland soils under anaerobic conditions. Evidence indicating that atrazine and cyanazine were biodegraded was not as convincing.

PLANT STRESS RESISTANCE: APPLICATIONS OF BIOTECHNOLOGY. Carole L. Cramer, Dept. of Plant Pathol., Physiol., and Weed Sci., Va. Polytechnic Inst. and State Univ., Blacksburg, Va. 24061-0330. Plants have evolved complex mechanisms for surviving biotic and abiotic stresses. Technologies based on recombinant DNA and genetic transformation of plants provide new tools for understanding the molecular basis of stress resistance and new strategies for engineering enhanced resistance. We have cloned tomato genes encoding 3-hydroxy-3-methylglutaryl CoA reductase (HMGR), a major rate-limiting enzyme of isoprenoid synthesis. HMGR activity is highly induced during expression of disease resistance associated with the synthesis of isoprenoid phytoalexin antibiotics. Specific HMGR genes are differentially expressed during normal development and in response to wounding or pathogen challenge. We are currently developing procedures to generate transgenic tomato roots and plants to analyze stress-related HMGR gene regulation and promoter function and to address whether altered levels of HMGR affect disease resistance.

BIOLOGICAL NITROGEN FIXATION. Dennis R. Dean, Dept. of Anaerobic Microbiology, Virginia Polytechnic Institute and State University, Blacksburg, Va. 2406!. Biological nitrogen fixation is catalyzed by nitrogenase, a complex metalloenzyme composed of two component proteins, called the Fe protein and the MoFe protein. Together, these two proteins catalyze the ATP-driven six-electron reduction of dinitrogen to ammonia with the concomitant evolution of dihydrogen. Effort from this laboratory is focused on elucidation of the molecular mechanism of biological nitrogen fixation. Towards this end we have isolated and characterized more than 35 genes whose products are known to be involved in the process. In addition, we have developed gene and site-directed mutagenesis strategies for elucidating the specific biochemical functions of the individual gene products. Finally, we have used site-directed mutagenesis techniques and biochemical and biophysical characterizations of altered nitrogenase component proteins to identify residues required for its catalytic activity.

SECONDARY METABOLITES. Walter Niehaus, Department of Biochemistry, V.P.I., Blacksburg, VA 24061. Secondary metabolites are compounds which have complex chemical structures, are biosynthesized by a limited group of organisms, and which are not required for the growth of the producing organism. Indeed, in most cases we do not know the physiological role played by the secondary metabolite in the life of the producing organism. Nevertheless, secondary metabolites are of great importance to mankind, as this class of compounds includes most of our antibiotic drugs, several anticancer drugs and cholesterol-lowering drugs, as well as a number of highly toxic or carcinogenic agents. The role of biotechnology is to introduce modifications into the producing organisms in order to increase yields of desirable secondary metabolites, or to decrease or eliminate production of undesirable secondary metabolites. In this talk I will consider two secondary metabolite groups: the β-lactam antibiotics such as penicillins and cephalosporins; and the polyketide mycotoxins such as aflatoxin and sterigmatocystin. I will briefly describe biotechnological approaches that have been successfully applied by scientists at Lilly to increase production of the antibiotics. I will discuss the current state of biotechnological approaches to the mycotoxin problem, including some work that has been done in my laboratory.

ANIMALS AS BIOREACTORS. Tracy D. Wilkins, Dept. of Anaerobic Microbiology, Va. Tech Blacksburg, VA. Recombinant DNA techniques are now used to produce simple human proteins in bacteria and yeast. Many other human proteins are so complex that only mammalian cells can produce them correctly. A multidisciplinary team at Va Tech is now attempting to produce such proteins in the milk of farm animals. Human genes controlled by mouse regulatory DNA have now been inserted into the chromosomes of pigs. These pigs are now being milked to determine whether the human proteins have been successfully expressed in the milk. If these experiments are successful many lives will be saved by the use of these proteins - which will be purified from the milk and injected into patients who require them.

GENOMIC ORGANIZATION AND EXPRESSION OF THE OVINE INSULIN-LIKE GROWTH FACTORS. Eric A. Wong and Susan M. Ohlsen*, Dept. of Animal Science, Va. Polytechnic Inst., Blacksburg, Va. 24061. The insulin-like growth factors (IGFs) are small peptides which are structurally related to insulin and possess growth-promoting activity. IGF-I is a 70 amino acid peptide which is thought to be the major mediator of the biological effects of growth hormone and therefore is a key regulator of postnatal mammalian growth. IGF-II is 67 amino acids in length and is postulated to act as a fetal growth regulator. To study the molecular regulation of the IGFs in ruminants, we have cloned complementary DNAs (cDNAs) encoding ovine IGF-I and -II. The ovine IGFs show greater than 92% amino acid identity with other mammalian IGFs. The expression of messenger RNAs (mRNAs) encoding ovine IGF-I and -II is complex. mRNAs containing different first exon sequences have been identified and are presumably generated by alternative RNA processing. The gene for ovine IGF-I has been cloned and partially sequenced. Ovine IGF-I contains five exons and spans greater than 50 kilobases of DNA. Current research efforts are focused on a molecular analysis of the regulatory regions which control the expression of these important growth factor genes.

USE OF ANTHER DERIVED HAPLOIDS IN POTATO BREEDING. Richard E. Veilleux, Dept. of Horticulture, Va. Polytechnic Inst. and State Univ., Blacksburg, Va. 24061. One of the major contributions of plant breeding in the twentieth century has been the development of hybrid cultivars of major crops, such as maize. Hybrids are only possible through cross pollination of selected homozygous inbred lines, usually obtained by self-pollination for several generations. For many crops, including potato, inbred lines are unavailable due to severe inbreeding depression or barriers to self-pollination. Through culture of anthers, the male reproductive organ of plants, immature pollen grains of selected genotypes can be induced to become embryogenic and develop into plants with the gametophytic or haploid chromosome number. By doubling the chromosome number of such haploids, the equivalent of inbred lines can be obtained within only a single "generation." We report the development of such androgenetically derived inbred lines of *Solanum phureja*, a cultivated South American potato species, and their breeding behavior in test crosses. The progenies of some anther-derived inbred lines were found to be superior for yield attributing characteristics than the original heterozygous plant from which they were derived, indicating considerable potential for this technique in potato improvement.