© 1983 Nature Publishing Group http://www.nature.com/naturebiotechnology

ASM TECHNOLOGY REPORT

APPLICATION OF RECOMBINANT DNA TECHNOLOGY TO CELLULOSE HYDROLYSIS

On March 7, 1983, a seminar at the American Society of Microbiology in New Orleans brought together, perhaps for the first time, scientists involved in the cloning of cellulase genes. One common objective of these scientists is to clone the cellulase genes into an organism so that cellulose hydrolysis and ethanol production occur simultaneously in one organism. Another objective is to enhance cellulase production. Most fungal cellulases, such as the cellulase from *Trichoderma,* take 100 to 180 hours to produce the enzyme in batch culture. This symposium highlighted three different techniques to clone the cellulase gene complex into *Escherichia coli.*

Using mRNA as a probe, Dr. Vern Seligy (National Research Council, Ottawa, Canada) summarized his group's work in cloning cellulase genes from *Schizophyllum commune.* He hybridized mRNA from induced (by cellulose) and noninduced cells and eliminated all nonessential materials. A cDNA strand was constructed, ligated into pBR322, and inserted into *E. coli.* Seligy and his associates then identified gene products by reaction to an antibody which was sensitive to the cellulase. The enzyme was reported to be expressed in small quantities and its activity was limited.

Using similar techniques with induced and non-induced cells enriching the mRNA and making cDNA, Darrel Stafford (University of North Carolina, Chapel Hill, NC) reported inserting mRNA into a phage recombinant system. After cloning this into *E. coli,* he did not observe cellulolytic activity. He also used synthetic oligonucleotides compatible with terminal amino acid sequences of cellobiohydrolase I as a probe and extracted the mRNA. Stafford then assayed for *in vitro* expression using a reticulocyte system but found no identifiable activity of the cellulase in *E. coli.*

David Wilson (Cornell University, Ithaca, NY) reported that he was able to achieve expression using *Thermomonospora* endocellulase cloned into *E. coli.* Wilson claimed that after the product was extracted, it maintained thermal stability (see news item pg. 139). Wilson's experiment is, significantly, one of the rare occasions when a gene cloned from a gram positive organism was expressed into a gram-negative organism.

Douglas Eveleigh (Rutgers University, New Brunswick, NJ) summarized some of his group's work on identifying vectors in *Zymomonas.* After constructing a shuttle vector for *Zymomonas* and *E. coli,* his group is now ready to construct a gene bank from cellulolytic bacteria and insert genes into *Zymomonas.* Finally, Brad Snedeor (Genentech Inc., San Francisco, CA.) explained some work at Genentech using the IS*120* insertion sequence from *Clostridium thermocellum.* He was able to put the IS*120* insertion sequence into *E. coli* and mutate the chromosomes. This is the first step in establishing a genetic system for *Clostridium,* an organism which could have practical application for conversion of cellulose.

Bland S. Montenecourt, Ph.D., is an associate professor at Lehigh University, Bethlehem, PA. She was the organizer of the ASM session interpreted above, Application of Genetic Engineering to Cellulose Hydrolysis.

PLANT RESEARCH

MICROPROPAGATION ENTERS A NEW COMMERCIALIZATION PHASE

Many plants can be increased in number by removing parts of their structures, and by growing these pieces into completely new plants. This technique, called vegetative or clonal propagation, probably started over 8,000 years ago with multiplication of wine grape selections[1]. Vegetative propagation continues to be of great horticultural importance, especially for increasing stocks of many fruits, flowers, trees, and vegetables. Vegetative propagation ensures reproduction of plants with desired qualities. Its practice usually results in a high degree of uniformity among plants and their products.

Even though varieties of different vegetative propagation methods have been developed by nurserymen, they all depend on using relatively large, macroscopic pieces of the parent plants. Pieces, such as buds or stem cuttings, may harbor diseases and pests. Also, for many important species, use of large plant pieces results in slow multiplication rates. Some species cannot be propagated at all by current conventional propagation methods. For some others, only juvenile plants, whose agronomic characteristics cannot be properly evaluated, respond to conventional propagation techniques.

Over the past 20 years, a completely new approach to vegetative propagation, called micropropagation, has emerged. In micropropagation, small, even microscopic pieces of the parent plant are multiplied by plant tissue culture technology. Nutrient media sustain the growth of these tissue pieces in aseptic and artificially controlled environments. Micropropagation methods differ from traditional methods of vegetative propagation primarily in the conditions under which the propagation is accomplished, but not in principle.

Plants can be micropropagated by microcuttings, by initiation of adventitious buds, and by embryogenesis. With microcuttings, small shoot pieces are cultured. By alteration of the culture medium and cutting process, the buds in the axils of the leaves are encouraged to grow and to form new shoots. The shoots can be subsequently rooted and planted. In the second micropropagation method, adventitious buds are initiated either directly from plant tissues, such as leaves, petioles, and roots, or indirectly via callus produced from these same tissues. Buds then form shoots, which can be rooted by varying the medium constituents. Embryogenesis, the third micropropagation route, emulates the normal development of an embryo in a seed, except for the sexual process of fusion of gametes. The zygote, in this case, consists of somatic cells of the parent plant. Provided there are no changes in the cell's genome, plants derived by embryogenesis are identical to the parent plant.

The first commercial applications of micropropagation were based on the discovery in the 1960s that *Cymbidium* orchids could be efficiently multiplied by meristem culture[2]. The tissue culture of apical or axillary bud meristems leads to the formation of a large number of protocorms. Within a few weeks, each protocorm can be divided into pieces and subcultured to produce many more protocorms.