MEETING REPORT

CORNELL SCIENTISTS CLONE, EXPRESS CELLULASE GENES IN E. COLI

NEW ORLEANS, Louisiana—The use of biomass feedstock was highlighted at the 83rd Annual Meeting of the American Society of Microbiology, held here on March 6–11. David B. Wilson and coworkers at Cornell University, Ithaca, NY, announced success in cloning one of the cellulase genes into *Escherichia coli*, with secretion of the enzyme into the medium. This represents an important step in the conversion of cellulose feedstocks to a variety of commercial products, such as chemicals and fuels.

Wilson's group constructed a clone bank from *Thermomonospora*, a thermophilic *Actinomyces* that grows at 65°C. It was originally isolated by a group at General Electric and, according to Wilson, "has one of the most active cellulase complexes found." Wilson then targeted endocellulase, one of the enzymes of the cellulase complex, for study. Wilson's group then isolated the endocellulase gene and mixed it with the plasmid pBR322 in the presence of *E. coli* strain Hb101. They obtained about 6 000 transformants, 2 500 of which were tetracycline resistant, indicating expression of the pBR322 plasmid. These clones were then screened for cellulase activity.

A restriction map of one transformant, D316, showed the presence of an 8.3 kb segment of *Thermomonospora* DNA in addition to the pBR322 DNA. Another contained an insert of only 6 kb. Wilson concluded that the smaller fragment is too short to code for the entire *Thermomonospora* endocellulase, which has a molecular weight of 94 000 dal; the insert could only code for a 65 000 dal polypeptide. Wilson proved that he cloned the *Thermomonospora* endocellulase into *E. coli* by preparing an antibody inhibition test against the cellulase. As expected, the assay showed total inhibition of the cellulase.

Wilson was surprised to discover that the enzyme was secreted into the medium by *E. coli*. He found roughly 30% of the activity in the supernatant, about 35% in the periplasm, and the rest inside the cell. Wilson showed that this was not due to leakage of the cellulase from the cell by correlating

the cellulase activity with that of a known periplasmic enzyme, alkaline phosphatase.

Wilson also reported that the enzyme was not produced very well in exponentially growing *E. coli*; synthesis started after growth of the microbe ceased. Cellulase produced by the host *Thermomonospora* is made throughout its growth cycle.

Wilson admitted that he had a "one in a million" chance of obtaining

expression of the gene. *E. coli* produced only about 1% (5–10 micrograms/liter) of the cellulase activity found in *Thermomonospora*. Wilson's results are not sufficient to permit scientists to convert cellulose to commercial products since hydrolysis is dependent upon expression of the entire cellulase complex. Nevertheless, Wilson's work represents an important step in cloning cellulase genes into faster growing cells. —Marcel Faber

CANADIAN WOOD-TO-ETHANOL PROJECTS ENTER PILOT STAGE

OTTAWA—The first Canadian plants to demonstrate the feasibility of producing ethanol and chemicals from biomass will begin operation this year. This comes at a time when industry and the government firm up their commitments to develop biomass conversion as a major Canadian industry.

Although the Federal government has a funding finger in every bioconversion pie, the most rapidly advancing

programs are being run by private corporations. Iotech, Stake Technology, and George Weston Ltd. (working with an Ontario Crown Corporation to form Bio-Hol) each expect to have pilot plants operating within the year. Other efforts, such as Allelix and the publicly funded Canertech and Forintec, are still definitely in the early stages of their research and development programs.

All of the bioconversion processes

Bio-Hol hopes its wood-to-ethanol fermentation process will prove to be an economical source of energy production. R&D of the wood-hydrolysis process will not be completed until the end of this year.