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Session 6B

Plant Biotechnology and Feedstock Genomics

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Over the first part of the twentieth century, it is expected that a large increase in the utilization of renewable bioresources will occur. The possibility for change arises from the broad range of research projects that are currently under way, and the drivers for change continue to include sustainable economic growth and national energy security, and to minimize anthropogenic effects on the environment.

The recent research focus on conversion of conventional lignocellulose biomass will provide useful results in clarifying potential application within various processing mills. However, additional technical approaches will also contribute to successful implementation of the overall biomass platform. For example, if the raw input material were to be "designed" to be more easily converted to a sugar platform, then the system efficiency, practical application, and economic viability would all increase. The recent significant advances in genomics and biotechnology applications provide a new opportunity to utilize renewable resources in ways that will help ensure sustainable enterprises.

The knowledge and understanding of genome sequences, gene function, gene expression, protein interactions, and metabolic control mechanisms will form the basis for future biotech-based alterations in primary production, which is the key underlying renewable resource. The participants in this session provided an exciting view, covering state-of-the-art developments in several relevant areas. Details are provided in the individual papers and an overview of the session is as follows:

Nathan Lakey (Orion Genomics) introduced the exciting capability to uncover the sequences of genes within the genome, without the need to sequence all the nongene DNA space. The technique takes advantage of a chemical difference between repetitive junk DNA, which is methylated and comprises ~90% of plant genomes, and genes, which remain unmethylated and occupy only 10% of the genome. This approach has been demonstrated in all of the major plant species and is estimated to provide the "genespace" sequence at a 10-fold lower cost and much faster than previous methods. The ultimate application to plants used for bioenergy would provide an operational base for significant improvements, primary

productivity, and composition, leading to much lower cost of input "sugars" and easier bioconversion to intermediates of interest.

Mike Lassner (Verdia Inc.) presented examples of the usefulness of directed molecular evolution as an in vitro process that more easily achieves what was traditionally attempted via reproductive crossing and recurrent selection (plant breeding). Proteins may be engineered that have specific desirable characteristics via methods that "evolve" the basic underlying DNA. For example, the outcome can be enzymes with improved kinetic properties that result in enhanced primary production, or proteins that remain operational under extreme conditions. In addition, compositional proteins may be enhanced to provide functional performance that was not achievable via conventional methods.

Justin Stege (Diversa Corporation) discussed the molecular evolution of enzymes for particular pathways, with a focus on the modification of oil composition. Oleochemical applications for such enzymes include applications as biocatalysts for fatty acid modifications. In a program to integrate production and processing, such enzymes can be used to modify the fatty acid content of vegetable oils *in planta*. Results show that expressing such new enzymes in oilseed crops has resulted in altered oil composition, and that the features may be used to better design plant-based oils for use as biofuels and as improved renewable feedstocks.

Mariam Sticklen (Michigan State University) presented results showing the successful expression of three different full-length polhydroxybutyrate (*phb*) genes in maize, and accumulation of the poly-(*R*)-3-hydroxybutyrate (PHB) enzymes within maize chloroplasts. PHB is a potentially useful polymer for use in the plastic markets and is currently produced via fermentation. PHB from transgenic plants was first demonstrated using Arabidopsis, but expression in major crops has added economic implications. This paper also reported work on codon-optimized *Trichoderma reesi cbh1* and wild-type *Acidothermus cellulolyticus e1* genes regulated by the rice *rbcS* promoter. Many maize plants were transformed with evaluations of protein targeting to various subcellular organelles.

Steve Thomas (National Renewable Energy Labortory) described the use of near-infrared (350–2500 nm) reflectance spectroscopy as a genetic screening tool to identify individual plants with particular cell-wall compositions. A broad range of corn genotypes was evaluated, including mutant populations, and the chemical composition was aligned with the phenotypic characteristics. Quantification of plant cell walls is an essential component if lignocellulose biomass is to become a major contributor to a biobased economy. Through such measurements it will be possible to better match biocatalysis to input composition, and to enhance raw material inputs through modified composition with more desirable characteristics.

Deborah Samac (USDA-ARS, Minnesota) reported that more than 100 genes are involved in cell-wall biosynthesis, and that many of these genes have now been cloned to better understand how modification could be achieved. Experiments on alfalfa involving the expression of a soybean

UDP-glucose dehydrogenase cDNA under the control of two promoters active in vascular tissues showed good expression in greenhouse tests. Other results provided evidence that altering the expression of a single gene may have only minor effects. Multigene changes or finding specific regulatory genes may be required to improve the ability to modify plant cell walls to have more useful features.

Overall the participants in this session covered a wide range of topics and clearly demonstrated the importance of genomics and biotechnology to the future of renewable resources. The major underlying threads seemed to be the following:

1. Rapid advances in the technology are opening the door for novel opportunities.
2. Enzymes can be improved via molecular evolution, and for use *in planta*.
3. Single gene changes may not be enough for coordinated pathway engineering.
4. Compositional quantification is important for baseline and useful modifications.

Above all, there was general agreement that the integration of these approaches may be much more productive than just exploring limited single areas. The true biorefinery concept requires integration of all the available science—to enable a broad range of input types along with an output portfolio that includes biofuels and a selected range of economically acceptable biobased products.

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