NATIONAL INSTITUTE OF SCIENCE AND TECHNOLOGY OF BIOETHANOL

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In the 70s, Brazil started a program to substitute gasoline by ethanol in order to decrease dependence from politically and economically variable periods. The plant species chosen was sugarcane and as a consequence, agricultural and technological studies were greatly intensified, leading Brazil to a very favorable position in terms of energy security. Nowadays, Brazil has more than 80% of its cars running with bioethanol and even airplane engines are now being developed. With the increasing political instability in the Middle East, since 2001, the USA has also decided to direct its energy policy towards the use of biofuels. This is now being followed by Europe and Japan and it is likely to be followed by several other countries in the world. This imposes an enormous pressure on the production of crops that can supply bioethanol.

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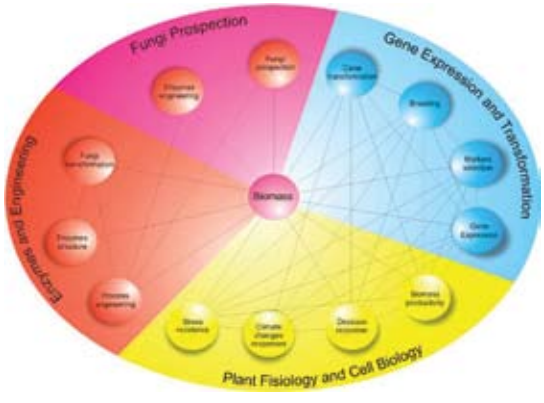

Figure 1. Structure of the INCT do Bioetanol, which is divided in 4 centers that congregate 30 laboratories in 6 states of Brazil

Brazilian sugarcane is probably the most efficient extant bioethanol producing system. However, only part of the biomass produced is used for bioenergy production, 1/3 of the plant being used for sucrose production, 1/3 is bagasse, which is burnt for production of electricity and the last third is left in the field and latter on decomposed by microorganisms. Therefore, in order to supply wider needs, a significant increase in production of ethanol is possible if we can provide the basic knowledge necessary for development of technologies that will be capable to obtain energy from the polysaccharides of the cell wall, which makes 70% of the biomass burnt inefficiently and left in the field. The availability of such processes within the distillery and the higher marketing value of liquid fuel provide additional economical advantages to its conversion instead of simply burning bagasse. Although the chemical hydrolysis of biomass is a consolidated methodology under laboratory conditions, its large-scale application is not economically viable, yet. The necessary use of acids reduces the life-time of equipments, produces toxic wastes and produces non-fermentable sugars, increasing the costs of the products. One alternative is the enzymatic hydrolysis of the cell wall. Such a process requires the use of a complex machinery of specific enzymes that are produced either by the plant itself or by microorganism able to degrade plant cell walls. On the other hand, relatively little is known about the structure and architecture of the cell wall. One of the goals of the INCT do Bioetanol (*Figure 1*) is understand the fine structure of the principal hemicelluloses of sugarcane and other possible sources of biomass. We intend to find patterns of gene expression that could be useful to find ways to induce the plants to degrade their own wall and become prepared for subsequent hydrolysis. In parallel, we intend to prospect microorganisms, enzymes and genes both in microorganisms and sugarcane, that are capable to efficiently hydrolyze the walls. Such enzymes will be designed to have the highest possible performance to degrade plant cell walls, especially the walls of sugarcane. A group of researchers screen existent varieties of sugarcane to find gene markers that could guide the groups to quickly identify plant materials that would be more suitable for use in industrial processes. From the latter viewpoint, our group intends to perform tests of mechanical preparation of sugarcane for further acid and/or enzymatic hydrolysis. With this data in hands, we expect to be able to provide the fundamental knowledge of biotechnology that is necessary for scaling up studies and further increase in efficient of bioethanol production in Brazil.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

UNDERSTANDING HOW SUGARCANE PLANTS FUNCTION

1) Sugarcane has been deeply studied regarding its physiological traits so that the latter could be related with the genetic markers under development by breeders and help to find superior sugarcane varieties in many senses. To do that, plant physiologists started to construct a databank that will be available to other researches;

2) To understand how sugarcane will respond to the global climatic changes, plants have been grown in elevated CO2 and it was found that its photosynthesis and biomass increased considerably. Researchers discovered that plants capture more light to compensate elevated CO_2 and activate genes related to the electron transport system. Now researchers will try to increase gene expression of photosynthesis to see whether growth is positively affected in the same way, but without the extra CO_2;

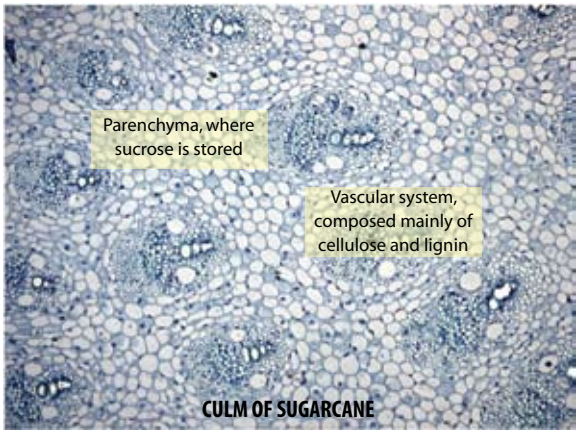

Figure 2. Section of the culm ("stem") of sugarcane showing where sucrose and walls are in the tissue. This picture shows the general structure of what has to be degraded by enzymes to produce free sugars for fermentation and production of bioethanol

3) With the development of molecular markers, it has been possible to map the genome so that researchers are starting to find genes that can indicate important features related to the agronomic features of sugarcane, such as higher productivity, resistance to drought, higher sugar and fiber contents;

4) The expression of important genes and proteins, related to photosynthesis, drought resistance, sugar content and cell wall metabolism are being studied. This information, together with the physiological data, will be important to design strategies to understand how sugarcane plants function. This information can be of great help to breeders as they could produce varieties (or modify plants genetically) that will be more productive and better adapted to different environmental conditions throughout the country.

PRODUCING THE BASIC SCIENCE FOR THE CELLULOSIC ETHANOL

1) The sugarcane cell wall had its chemical structure determined and the polysaccharides have been subjected to hydrolysis with fungal enzymes to understand their mode of action;

2) Sugarcane tissues were sliced and analyzed anatomically (*Figure 2*). We have now enough data and are producing an Atlas that will permit researchers to understand plant better structure of the tissues that have to be degraded to produce bioethanol;

3) Sugarcane bagasse has been characterized and pre-treatment systems are under intensive focus, especially the use of acid hydrolysis and steam explosion;

4) The chemical structure of the trash left in the field has been followed for over a year and the quality of this material, important for use as raw material for second generation bioethanol, is now established;

5) Several fungi species have been found to produce enzyme cocktails capable to degrade sugarcane cell walls. Many enzymes of these cocktails have been purified, their genes cloned and heterologously expressed in bacteria;

6) Yeast species were found that are capable to metabolize pentoses such as xylose and arabinose.

7) Enzymes have been crystallized and some were engineered by artificially introducing catalytic sites from laccase and xylanase in the same protein. These enzymes are being tested with different substrates.

MAIN PUBLICATIONS

Buckeridge MS, Santos WD, de Souza AP 2010. Routes to cellulosic ethanol in Brazil. In *Sugarcane Bioethanol: R&D for productivity and sustainability* (Cortez LB Editor. In press. ISBN 978-85-212-0530-2

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