It's time for biotechnology in biomass

GranBio invests in R & D to meet the challenges of producing second generation ethanol

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Pereira, standing at left, and the GranBio research and development team in Campinas. In the rear, bales of straw from sugarcane

After sugarcane is harvested, the remaining straw is the principal raw material GranBio uses in ethanol production. In September 2014, the company became the first in Brazil to produce cellulosic ethanol or second-generation (2G) ethanol, which is made from biomass by an industrial scale biotechnological process. Traditionally, first-generation ethanol fuel is produced from sugarcane juice. GranBio, an industrial biotechnology company founded in 2011 and headquartered in the capital city of São Paulo State, operates over the entire length of the supply chain. GranBio also pursues scientific and technological solutions for several components of the agricultural and industrial production system.

The company has already made important technological strides, such as constructing genetically modified yeast and varieties of sugarcane aimed at producing 2G ethanol. The Bioflex 1 plant was set up in São Miguel dos Campos in the northeastern state of Alagoas next to the Caeté Plant, which produces first-generation ethanol. GranBio obtains sugarcane straw from the Caeté Plant and three other plants in the region. Cellulosic ethanol is produced experimentally in only a few commercial plants around the world. Two commercial plants in the United States use the stems and leaves of maize as a raw material, and one in Italy uses wheat leaves. In July 2015, the Brazilian energy company Raízen opened a plant in the city of Piracicaba, São Paulo State that uses sugarcane bagasse and straw.

COMPANY PROFILE

GRANBIO

R & D Center
Campinas,
São Paulo State
Nº of Employees
18
Main products
Development of technology for ethanol production from the leaves and stalks of sugarcane

LÉO RAMOS

GranBio's initial strategy was to import technology from abroad to speed up the production process. A number of ingredients were chosen, such as yeast from the Dutch company DSM and enzymes from the Danish company Novozymes. At the same time, GranBio set up a research and development (R&D) center in Campinas. "What we do is convert science into technology," says Gonçalo Amarante Guimarães Pereira, a GranBio partner and chief scientist at the company. At age 51, Pereira has been a professor at the Genomic and Expression Laboratory of the Institute of Biology of the University of Campinas (Unicamp) for 18 years. Most members of the research team he leads were recruited from the university. Currently, there are 18 researchers and technicians working directly on R & D: eight doctoral degree holders, two master's degree holders and four doctoral students.

SPECIAL YEAST

The team led by Pereira was responsible for two recently announced technological achievements. The first is the development of a yeast strain capable of processing xylose, the sugar present in hemicellulose, one of the three main types of fiber in sugarcane leaves and stems along with cellulose and lignin. "In the first generation, the industrial yeast strain (of the *Saccharomyces cerevisiae* species) consumes the sucrose and fructose found in soluble form in sugarcane juice to produce ethanol; in bagasse, the sugars in the fibers of the leaves, such as xylose and pentose, are not soluble and so the yeast does not recognize them," says Pereira. To make yeast capable of processing xylose, the GranBio team developed a genetically modified strain with a gene from another microorganism—which they prefer not to reveal—and some modified genes of the *Saccharomyces* species itself.

The modified organism was approved for commercial use by the general coordination office of the National Biosecurity Commission (CTNBio) in April, and its use is the subject of a patent filed with the Brazilian Industrial Property Institute (INPI). "We will start using genetically modified yeast on the production line in 2016." The use of xylose, according to Pereira, produces a profit of approximately R$50 million annually for the company, which intends to

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1 In Pauliceia (São Paulo State), on the left, traditional sugarcane and, on the right, energy-sugarcane

2 Sugarcane straw in the molecular biology laboratory

3 Sugarcane samples in the analytical chemistry laboratory

4 Ethanol test in a biochemical analyzer

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process 400,000 metric tons of biomass per year. "Glucose makes up 40% of this material, pentose approximately 35%, and xylose 25%, which translates into approximately 100 metric tons. For the second generation to be profitable, xylose and other sugars found in the straw and bagasse must be processed."

The yeast genetic engineering work was led by biologist Leandro Vieira dos Santos, 32. "We did a study to identify genes and combinations of genes that would induce *Saccharomyces* to consume xylose," says Santos, who is working on a doctorate at Unicamp. Santos is a graduate of the Federal University of Viçosa (UFV), where he received a master's degree in microbiology. After working for two years at the biotechnology company Agrogenética, he decided to pursue a doctorate focusing on yeast. He contacted Pereira in 2011, when the researcher was putting together the GranBio team. Today, with the yeast ready, Santos is working on perfecting it.

The propagation of the microorganism and time scale are the work of biotechnology engineer Luige Calderon. He is originally from Peru, where he graduated from the Catholic University of Santa Maria in Arequipa. Calderon obtained a master's degree from Unicamp and, in 2012, was recruited to work as a researcher at GranBio in the bioprocess area. "I select microorganisms using genetic and evolutionary engineering, and then develop, for example, the most appropriate means of cultivation," Calderon explains. Yeast genetics also drew Osmar de Carvalho Netto to the company. He has a degree in food science from the University of São Paulo (USP) and a doctorate from Unicamp. He participated in the sequencing of the *Saccharomyces* genome at Unicamp and thought about forming an industrial yeast company with Santos. "We were going to be one of GranBio's suppliers, but they convinced us to join the company instead," he says. He became the company's process coordinator for areas such as fermentation, hydrolysis and pretreatment of biomass. He was also assigned to bridge the gap between research and corporate areas. "It had to be someone who understands the language of scientists and I now spend half my time on this. The other half is spent on process coordination," he says. For example, he is involved in organizing tests, delivering materials and staying in touch with the plant, all which is conducted so that GranBio's researchers can concentrate on research.

INSTITUTIONS FROM WHICH GRANBIO RESEARCHERS GRADUATED

Gonçalo Amarante Guimarães Pereira, Chief Scientist (agronomist)	Federal University of Bahia (UFBA) - undergraduate University of São Paulo (USP) - master's degree and post doctorate University of Düsseldorf, Germany - doctorate
José Bressiani, Agricultural Director (agronomist)	University of São Paulo (USP) - undergraduate, master's and doctoral degrees
Osmar Carvalho Neto, Process Coordinator (food scientist)	University of São Paulo (USP) - undergraduate and master's degrees University of Campinas (Unicamp) and University of Colorado, United States - doctorate
Leandro Vieira dos Santos, Researcher (biologist)	Federal University of Viçosa (UFV) - undergraduate and master's degrees University of Campinas (Unicamp) - doctorate (in progress)
Luige Calderon, Researcher (biotechnology engineer)	Catholic University of Santa Maria, Peru - undergraduate degree University of Campinas (Unicamp) - master's degree and doctorate (in progress)

BACK TO THE BEGINNING

In addition to yeast, another GranBio innovation was the energy-sugarcane introduced in August. This sugarcane is a new non-transgenic variety developed with traditional crosses between several other cultivars in collaboration with the University Network for the Development

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of the Sugar-Energy Sector (Ridesa) of Alagoas and the Campinas Institute of Agronomy (IAC). "We went back to the beginning of sugarcane breeding. Instead of increasing sugar in the juice, we boosted the amount of fiber in the plant. And so we have a more rustic sugarcane. It is taller, has harvest longevity, flowers less and is more disease-resistant as well as being harder," says José Bressiani, an agronomist and the company's agricultural director. Along with undergraduate, master's and doctoral degrees in breeding from USP, Bressiani gained experience in producing sugarcane cultivars during his 15 years working at the Sugarcane Technology Center (CTC) and five years at Canavialis, part of the Monsanto group. "My job is to think about the biomass function for sugarcane plants. We are creating a plant that should have low production costs," he says.

Tests with energy-sugarcane, which bears the trade name Cana Vertix, are taking place in the Southeast, Northeast and Central-West of Brazil. The idea is that, in the future, specific sugarcane varieties will be used only to produce 2G ethanol and to generate electric energy by burning waste. Initially, only the straw of Cana Vertix will be used. After mechanical harvesting of the sugarcane, the straw is left behind on top of the soil. It dries after a few days, after which it is either collected and transported to the 2G plant or stockpiled for several months.

As of August 2015, the company had produced 3 million liters of ethanol just from sugarcane straw

Technological advances and the growth of GranBio increased the importance of the R & D center, which is now a subsidiary known as BioCelere. The formation of BioCelere began in March of 2010 with a conversation between Pereira, Bernardo Gradin, president of GranBio, and Alan Hiltner, executive vice president of GranBio. Gradin was stepping down as president of Braskem and wanted to invest in biotechnology and 2G ethanol. "We were at a restaurant and on the back of the check I outlined what the future GranBio could do in the scientific field. He liked it and then invited me to be one of the partners," says Pereira. The two had met before—they served together in the Army in Bahia. They were reunited in 2007 when Pereira coordinated a project between Unicamp and Braskem on renewable propylene (raw material for the production of plastics) made with sugarcane for the Partnership for Technological Innovation (PITE) of FAPESP. GranBio's investment has already reached $265 million for a production capacity of 82 million liters per year. As of August 2015, ethanol production had reached a total of 3 million liters. It was not as high as we had hoped because some industrial processes needed improvement. "But we have already found solutions and are going to implement them in early 2016," says Pereira. ■

PHOTOS 1 GRANBIO 2, 3 AND 4 LÉO RAMOS