National Institute of Science and Technology of Bioethanol

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INCT BIOETANOL

FAPESP

PETROBRAS

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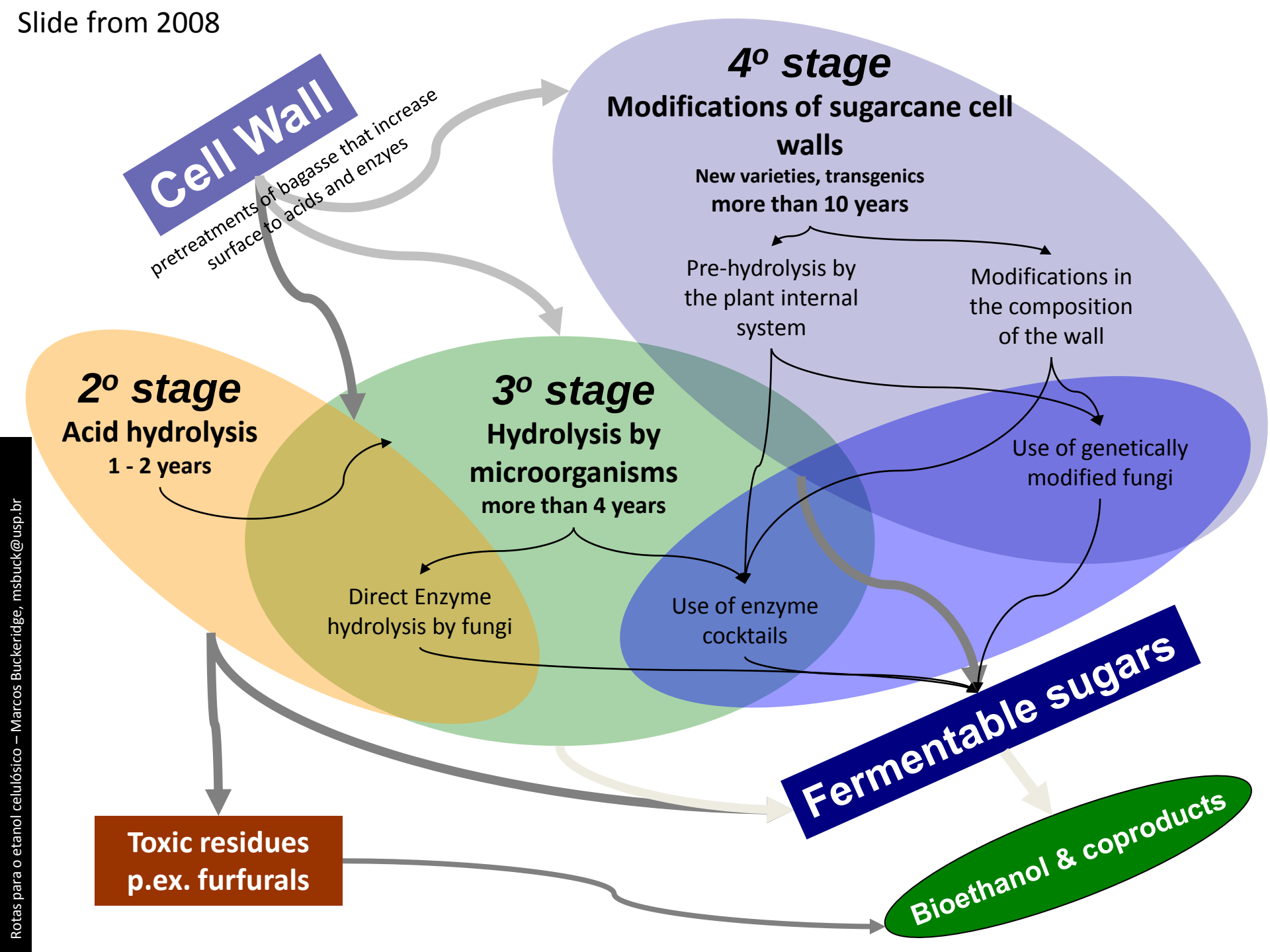
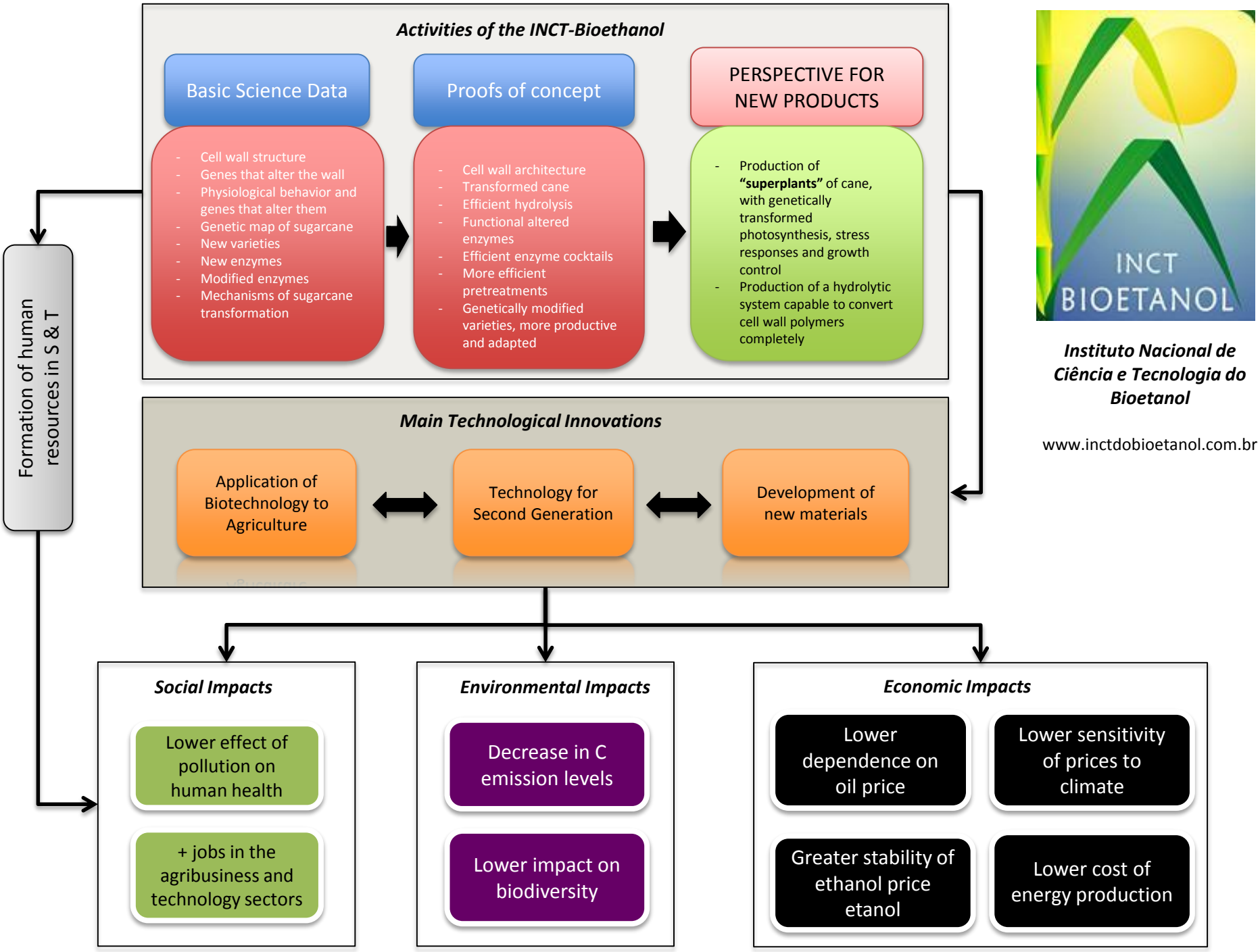
CAPES

CNPq Conselho Nacional de Desenvolvimento Científico e Tecnológico

Slide from 2008

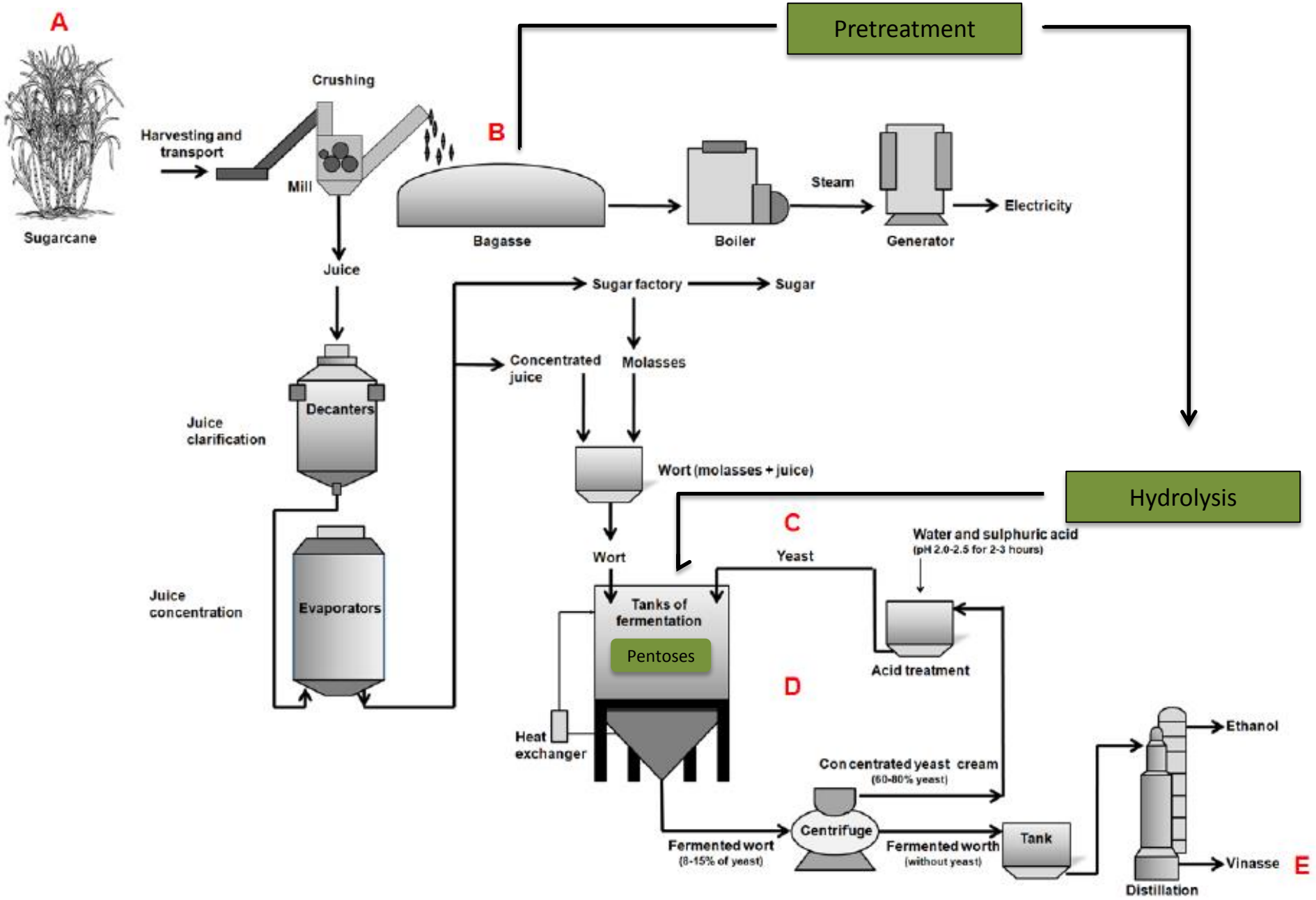


Rotas para o etanol celulósico – Marcos Buckeridge, msbuck@usp.br



Instituto Nacional de Ciência e Tecnologia do Bioetanol

www.inctdobioetanol.com.br



Amorim, Lopes, Oliveira, Buckeridge, Goldman (2011) Scientific challenges of bioethanol. Products in Brazil. Appl. Microbiol. Biotechnol. 91:1267

HIDRÓLISE

Growth of Rumen Bacteria on Plant Cell Wall Polysaccharides

1969

By R. T. J. CLARKE, R. W. BAILEY AND BLANCHE D. E. GAILLARD

Plant Chemistry Division, Department of Scientific and Industrial Research, Palmerston North, New Zealand

(*Accepted for publication* 2 *December* 1968)

SUMMARY

Three strains of *Butyrivibrio fibrisolvens* were isolated from the rumen contents of cattle feeding on red (*Trifolium pratense* L.) or white (*T. repens* L.) clover. The substrates used in these isolations were plant hemicellulose fractions other than simple insoluble xylan. The strains showed some differences in their ability to grow on various plant polysaccharides and to secrete polysaccharases specific to these polymers. The same type of rumen contents yielded, on polygalacturonic acid media, a strain of *Lachnospira multiparus* which grew only on pectin and secreted as sole polysaccharase a polygalacturonase. Only one of the three *B. fibrisolvens* strains grew vigorously on polygalacturonic acid and its polygalacturonase appeared to be different to that of the *L. multiparus*.

INTRODUCTION

The main polysaccharides of pasture plant cell walls are cellulose and xylan and many studies have been made on the action of rumen bacteria on these compounds. Thus strains of *Butyrivibrio fibrisolvens*, *Bacteroides succinogenes*, *B. ruminocola*,

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1998

FEBS 20032

A scheme for designating enzymes that hydrolyse the polysaccharides in the cell walls of plants

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Received 29 January 1998

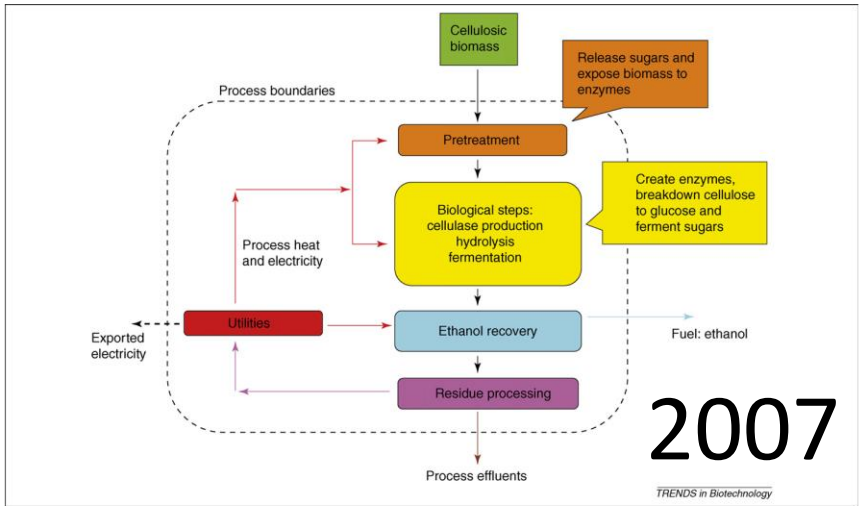
Abstract **A scheme is proposed for designating enzymes that hydrolyse the polysaccharides in the cell walls of plants. These enzymes are predominantly β-1,4-glycanases. The scheme is based on the classification of the catalytic domains of glycoside hydrolases into families of related amino acid sequences. The new designation for an enzyme indicates its family and, because all members of a family have these characteristics in common, its three-dimensional fold and stereospecificity of hydrolysis. The scheme is intended to simplify comparison of the systems of enzymes produced by different microorganisms for the hydrolysis of plant cell walls.**

Key words: Enzyme nomenclature; Structure; Glycosyl hydrolase

of these enzymes better than substrate specificity alone; (ii) help to reveal the evolutionary relationships between these enzymes; and (iii) provide a convenient tool to derive mechanistic information from the protein sequence data [7,8] (an updated list of the glycosyl hydrolase families can be found at the Expasy server http://www.expasy.ch/cgi-bin/lists?glycosid.txt). Many glycoside hydrolases are modular, comprising a catalytic domain (CD) and one or more ancillary domains [1,10]. The CDs catalyse hydrolysis with either retention or inversion of the configuration at the anomeric centre of the substrate [11–14]. In addition to revealing overall structural relationships of glycosyl hydrolases, the hydrophobic cluster analysis (HCA) and amino acid sequence alignment of the catalytic domains allow prediction of the stereospecific out-

Opinion TRENDS in Biotechnology Vol.25 No.4

What is (and is not) vital to advancing cellulosic ethanol



2007

Figure 1. Simplified process flow for biological conversion of cellulosic biomass to ethanol.

Metagenomic Discovery of Biomass-Degrading Genes and Genomes from Cow Rumen

Matthias Hess,[1,2]* Alexander Sczyrba,[1,2]* Rob Egan,[1,2] Tae-Wan Kim,[3] Harshal Chokhawala,[3] Gary Schroth,[4] Shujun Luo,[4] Douglas S. Clark,[3,5] Feng Chen,[1,2] Tao Zhang,[1,2] Roderick I. Mackie,[6] Len A. Pennacchio,[1,2] Susannah G. Tringe,[1,2] Axel Visel,[1,2] Tanja Woyke,[1,2] Zhong Wang,[1,2] Edward M. Rubin[1,2]†

The paucity of enzymes that efficiently deconstruct plant polysaccharides represents a major bottleneck for industrial-scale conversion of cellulosic biomass into biofuels. Cow rumen microbes specialize in degradation of cellulosic plant material, but most members of this complex community resist cultivation. To characterize biomass-degrading genes and genomes, we sequenced and analyzed 268 gigabases of metagenomic DNA from microbes adherent to plant fiber incubated in cow rumen. From these data, we identified 27,755 putative carbohydrate-active genes and expressed 90 candidate proteins, of which 57% were enzymatically active against cellulosic substrates. We also assembled 15 uncultured microbial genomes, which were validated by complementary methods including single-cell genome sequencing. These data sets provide a substantially expanded catalog of genes and genomes participating in the deconstruction of cellulosic biomass.

Biofuels derived from lignocellulosic plant material represent an important renewable energy alternative to transportation fossil fuels (*1*, *2*). A major obstacle to industrial-scale production of fuel from lignocellulose lies in the inefficient deconstruction of plant material, owing to the recalcitrant nature of the substrate toward enzymatic breakdown and the relatively low activity of currently available hydrolytic enzymes. Although the success of protein engineering to improve the performance of existing lignocellulose-degrading enzymes has been limited (*3*), retrieving enzymes from naturally evolved biomass-degrading microbial communities offers a promising strategy for the identification of new lignocellulolytic enzymes with potentially improved activities (*4*).

Metagenomics, the direct analysis of DNA from environmental samples, represents a strategy for discovering diverse enzymes encoded in nature (*5*, *6*). Although metagenomics has been used

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*These authors contributed equally to this work.
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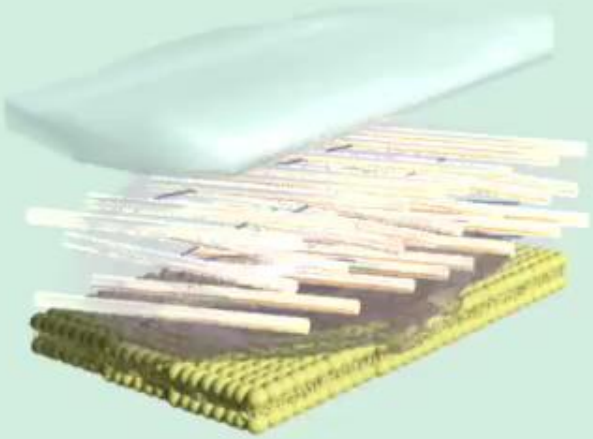
2011

Cell wall model

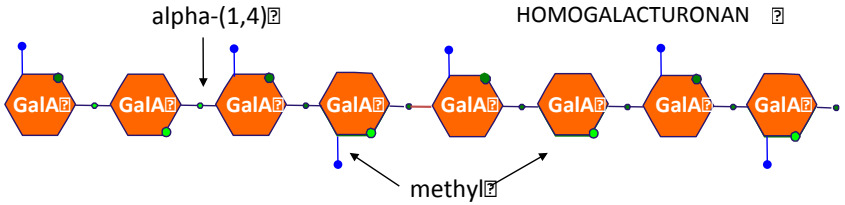


Arte: Monique Rached, Conceito: M.Buckeridge

CELL WALL POLYSACCHARIDES IN SUGARCANE

LINKAGES IN POLYSACCHARIDES
14 glycosidic
8 ester
*i.e. at least **24 linkages***

Pectins of sugarcane



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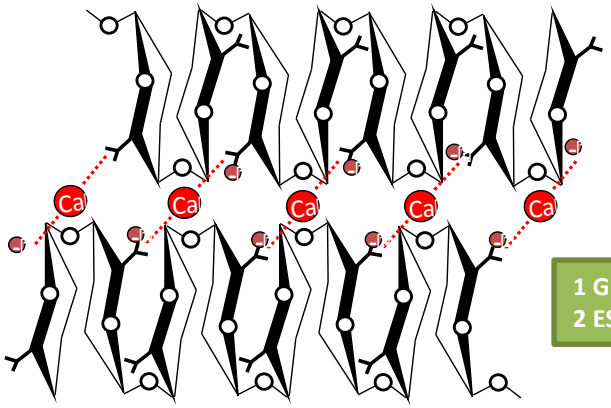
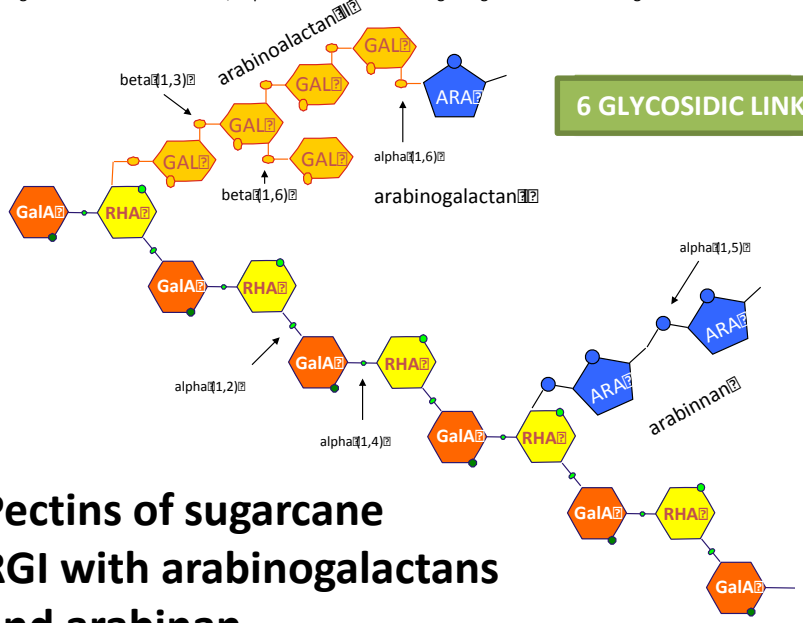


***Egg boxes** divalent ion, mainly calcium and magnesium induce the formation of gels in regions that are not methylated of homogalacturonan*

1 GLYCOSIDIC LINKAGE
2 ESTER LINKAGES (ACETYL AND METHYL)

B

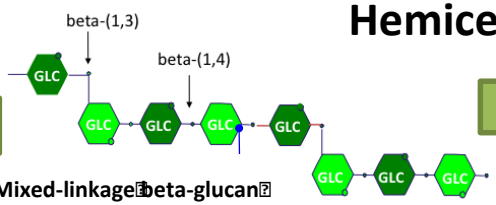
Buckeridge et al. 2008. Parede Celular, Cap 5 in Kerbauy G.B. Fisiologia Vegetal. Guanabara Koogan



6 GLYCOSIDIC LINKAGES

Pectins of sugarcane
RGI with arabinogalactans and arabinan

Hemicelluloses of sugarcane

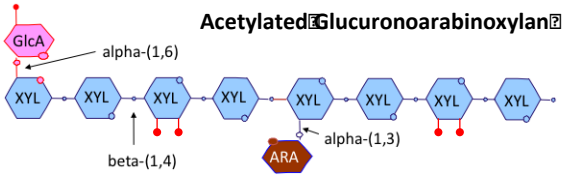


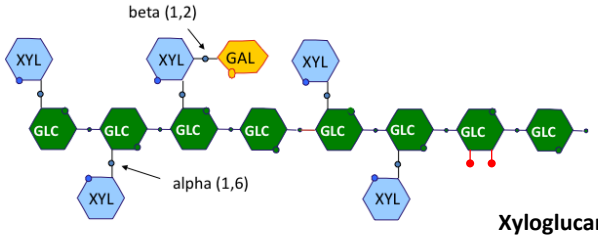
Mixed-linkage beta-glucan

2 GLYCOSIDIC LINKAGES

3 GLYCOSIDIC LINKAGES
2 ESTER LINKAGES
(ACETYL, FERULIC)

Acetylated Glucuronoarabinoxylan

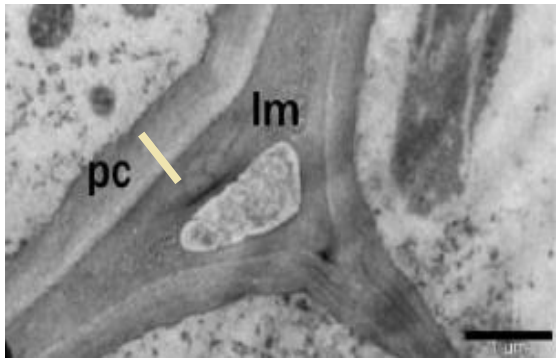




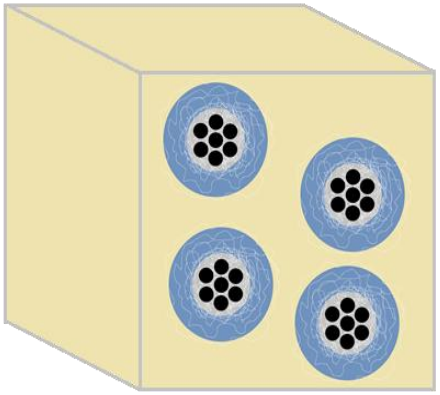
2 GLYCOSIDIC LINKAGEs
2 ESTER LINKAGES
(ACETYL, FERULIC)

Xyloglucan

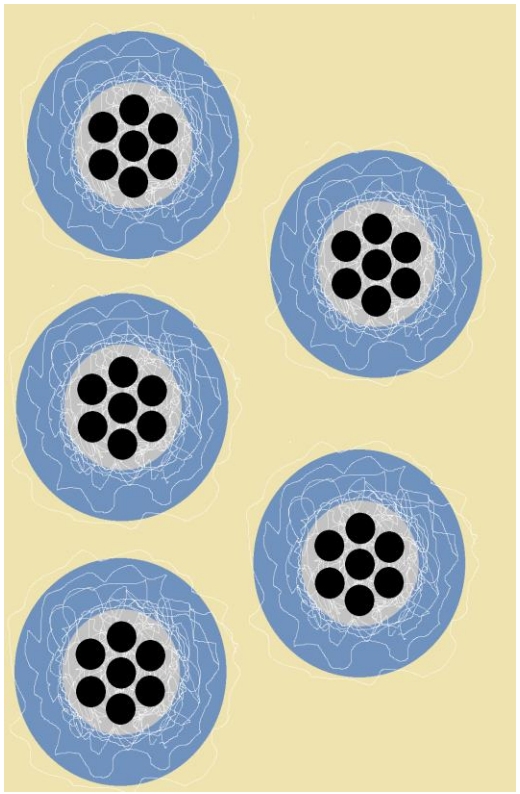
Cell Wall Architecture of grasses



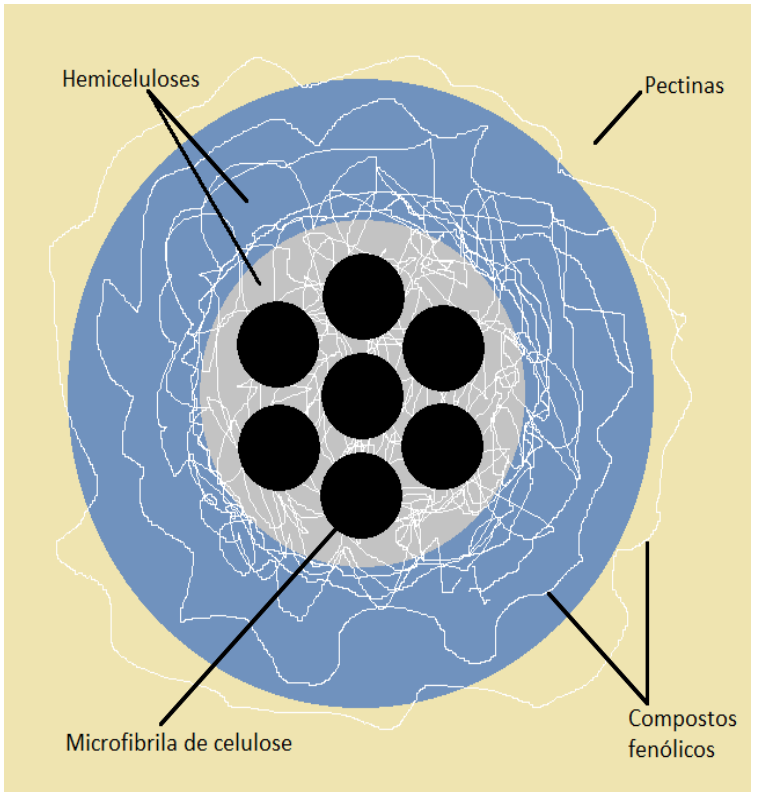
Sugarcane Cell Wall seen by TEM
(LEITE, 2013)



3-D representation of the Cell wall



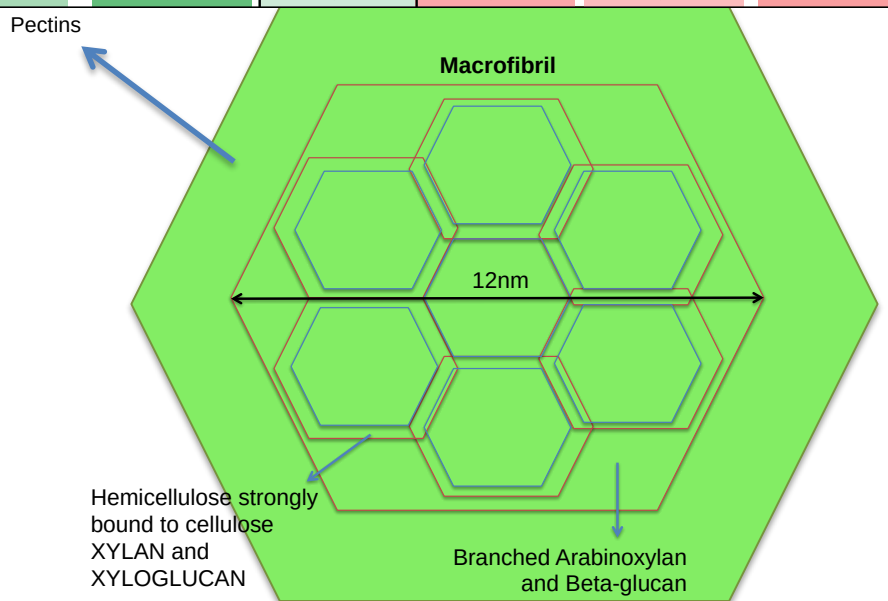
Cross section of the Cell Wall

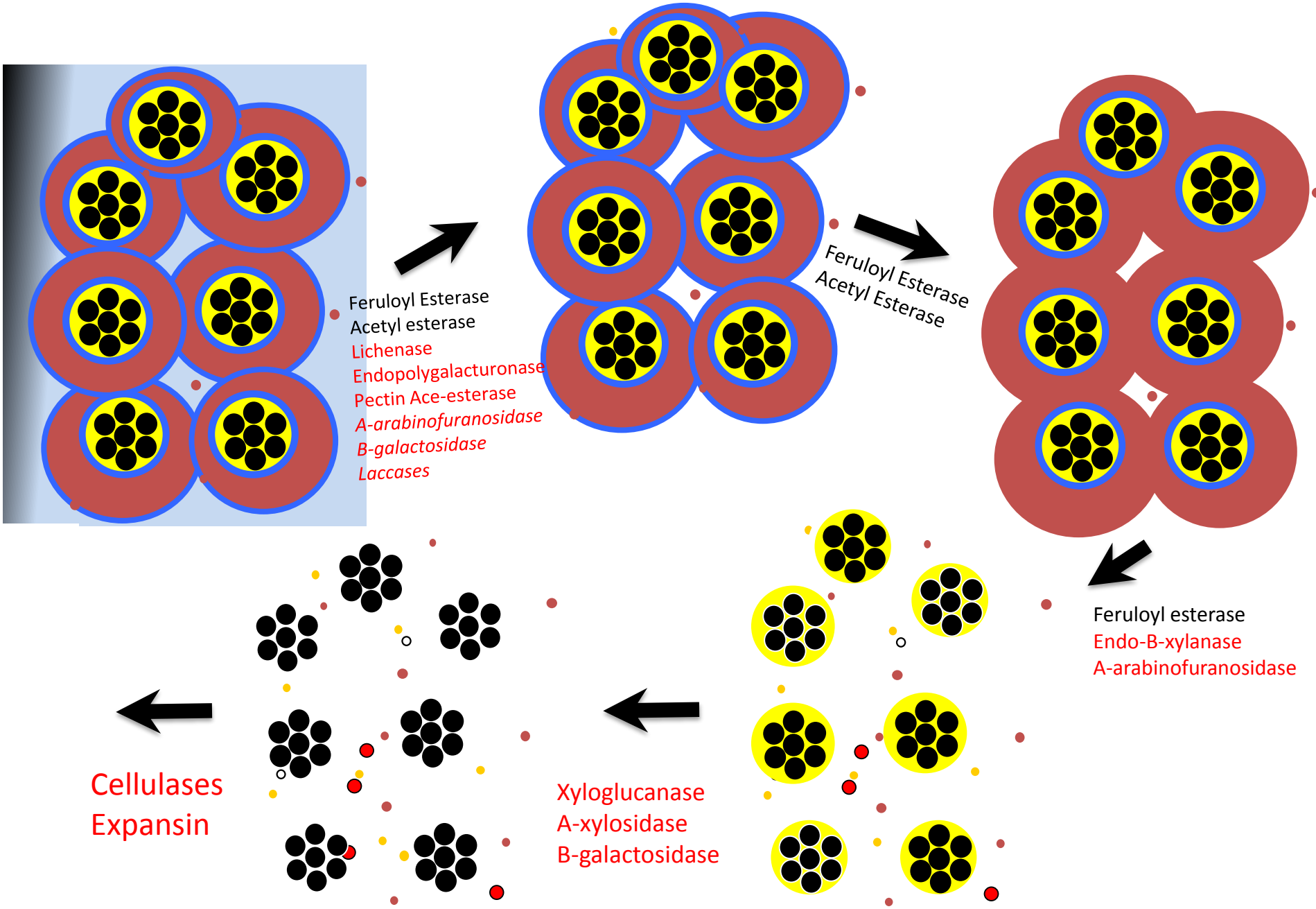


Detail of an Architectural Cell Wall Unit

KIT WITH 16 ANTIBODIES FOR SUGARCANE CELL WALL ANALYSIS

Antibodies	Pectins and loosely bound hemicelluloses						Strongly bound hemicelluloses			
	Ammonium Oxalate		Sodium Chlorite		0.1M NaOH		1M NaOH		4M NaOH	
	Culm	Bagasse	Culm	Bagasse	Culm	Bagasse	Culm	Bagasse	Culm	Bagasse
Xyloglucan	0,22	0,13	0,24	0,07	0,44	0,12	0,68	0,73	0,69	0,68
Xyloglucan	0,09	0,05	0,06	0,02	0,23	0,06	0,48	0,52	0,37	0,45
Arabinoxylan	0,89	0,82	0,47	0,36	0,62	0,48	0,65	0,68	0,64	0,73
Arabinoxylan	0,33	0,34	0,05	0,03	0,72	0,62	0,61	0,60	0,61	0,65
Arabinoxylan	0,82	0,68	0,37	0,53	0,63	0,57	0,55	0,64	0,50	0,48
Arabinoxylan	0,85	0,73	0,47	0,81	0,70	0,60	0,46	0,44	0,49	0,49
Arabinoxylan	0,96	0,94	0,88	0,93	0,67	0,46	0,63	0,63	0,71	0,73
Beta glucan	0,47	0,63	0,09	0,04	0,62	0,43	0,81	0,91	0,62	0,51
Homogalacturonnan	0,52	0,26	0,20	0,08	0,54	0,36	0,39	0,27	0,19	0,18
Homogalacturonan	0,57	0,12	0,13	0,02	0,02	0,03	0,04	0,01	0,04	0,04
Rhamnogalacturonan	0,71	0,55	0,43	0,18	0,56	0,26	0,43	0,32	0,39	0,33
Rhamnogalacturonan	0,60	0,26	0,33	0,09	0,40	0,14	0,19	0,14	0,11	0,12
Rhamnogalacturonan	0,56	0,24	0,27	0,07	0,33	0,12	0,16	0,13	0,10	0,11
Arabinoglactan	0,38	0,10	0,21	0,03	0,48	0,33	0,25	0,23	0,11	0,15
Arabinoglactan	0,66	0,45	0,24	0,05	0,14	0,05	0,07	0,04	0,04	0,04
Arabinoglactan	0,78	0,48	0,54	0,18	0,30	0,25	0,18	0,09	0,14	0,14





MODIFIED FROM: De Souza AP, Leite DCC, Patathil S., Hahn Mj, Buckeridge MS (2012) Composition and Structure of Sugarcane Cell Wall Polysaccharides: Implications for Second-Generation. Bioethanol Production. Bioenergy Research 6:564-579

In 2013

INCT Bioetanol was the Cover matter of the international issue of Revista Pesquisa FAPESP

More than 70 enzymes have been characterized and the cell wall of sugarcane has been unveiled

Journal of Experimental Botany
doi:10.1093/jxb/erv171

REVIEW PAPER

How endogenous plant cell-wall degradation mechanisms can help achieve higher efficiency in saccharification of biomass

Eveline Q. P. Tavares, Amanda P. De Souza and Marcos S. Buckeridge*

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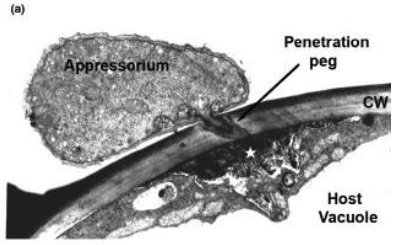
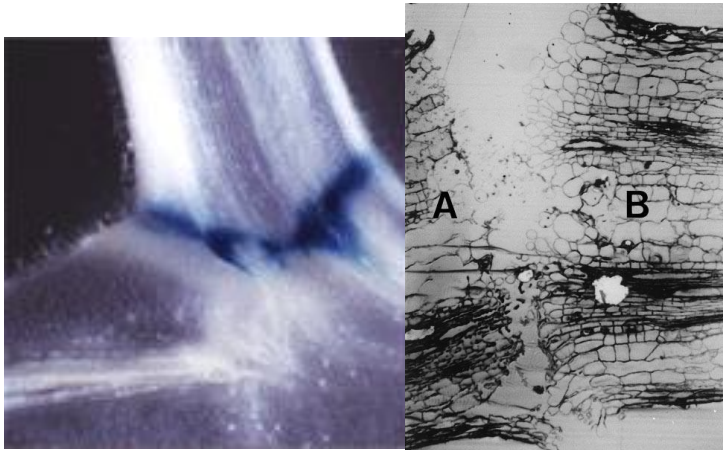
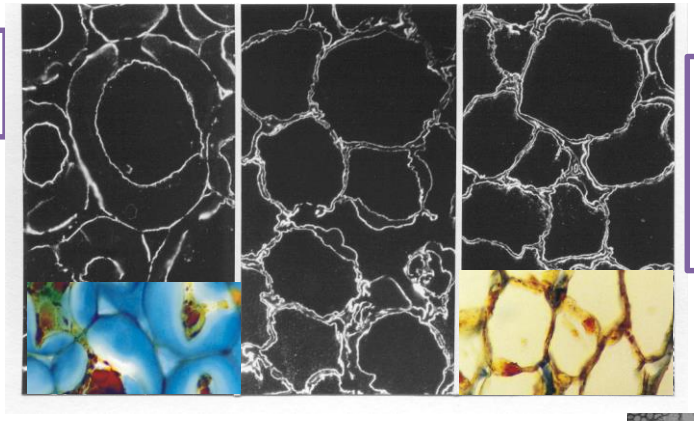
* To whom correspondence should be addressed. E-mail: msbuck@usp.br



Attack of microorganisms

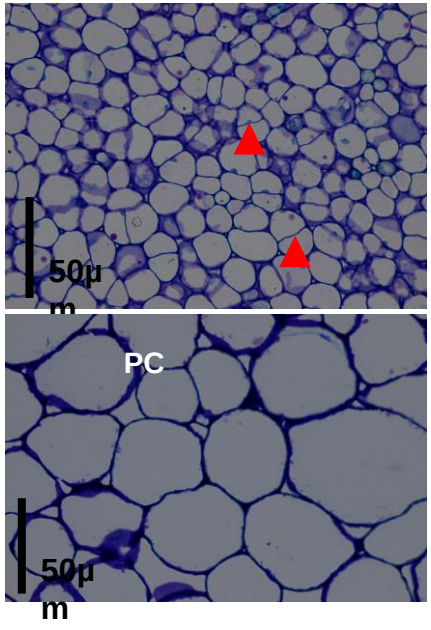


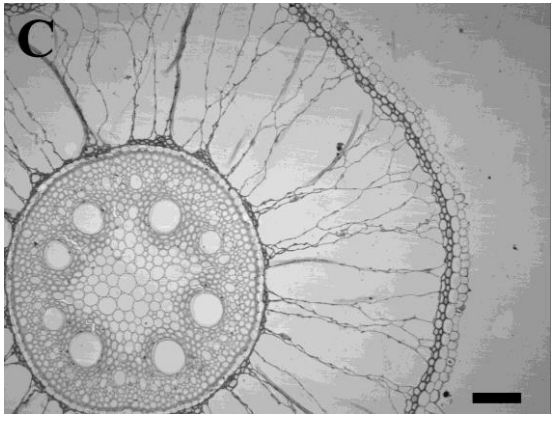
Leaf abscission



Storage Cell Wall Mobilization in seeds

Fruit ripening



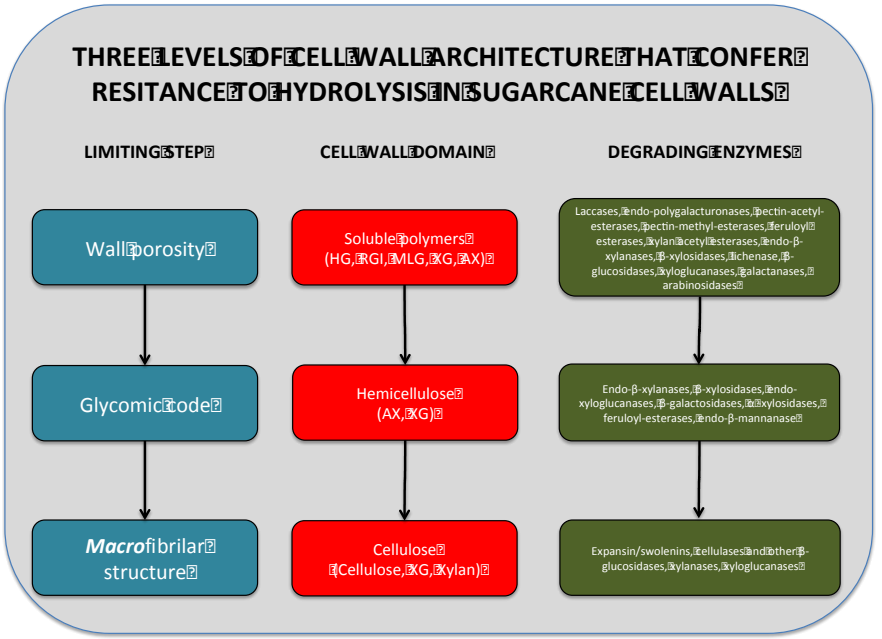


Aerenchyma development

RNAseq (unpublished)

48 genes of sugarcane to be used for cell wall disassembly

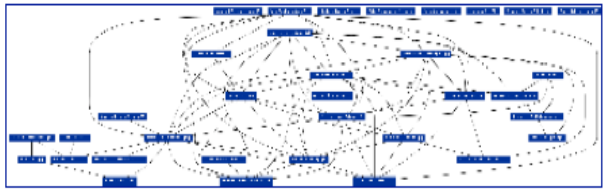
GENE	SEGMENT1	SEGMENT2	SEGMENT3	SEGMENT4
WALL POROSITY-RELATED ENZYMES	GENE EXPRESSION FPKM (log2)			
Glycoside hydrolase family 28 protein (polygalacturonase)	0.0	0.0	34.6	0.0
Glycoside hydrolase family 28 protein (polygalacturonase)	0.0	6.3	6.3	5.0
Glycoside hydrolase family 28 protein (polygalacturonase)	0.0	0.0	17.1	0.0
Glycoside hydrolase family 28 protein (polygalacturonase)	0.0	0.0	5.1	5.9
Glycoside hydrolase family 28 protein (polygalacturonase)	0.0	0.0	5.0	6.0
Pectate lyase	0.0	0.0	11.6	0.0
Pectinacetylesterase family protein	0.0	0.0	11.3	0.0
Plant invertase/pectin methylesterase inhibitor or Pectinesterase	0.0	0.0	16.6	0.0
pectinesterase family protein	0.0	0.0	17.8	0.0
Beta-galactosidase	5.4	7.4	7.1	7.1
Beta-galactosidase	0.0	0.0	17.2	0.0
laccase-like protein	0.0	8.5	7.8	0.0
phenylalanine ammonia-lyase (PAL)	0.0	8.3	8.7	0.0
phenylalanine ammonia-lyase (PAL)	11.2	14.2	14.1	6.9
phenylalanine ammonia-lyase (PAL)	4.7	5.3	6.4	5.6
GLYCOMIC CODE-RELATED ENZYMES				**15**
beta-1,3-glucanase Glycosyl hydrolases family 17	0.0	0.0	0.0	17.2
beta-1,3-glucanase Glycosyl hydrolases family 17	0.0	0.0	11.5	0.0
beta-1,3-glucanase Glycosyl hydrolases family 17	15.5	23.4	22.0	15.9
beta-1,3-glucanase Glycosyl hydrolases family 17	0.0	0.0	17.2	0.0
beta-1,3-glucanase Glycosyl hydrolases family 17	0.0	0.0	9.1	0.0
beta-1,3-glucanase Glycosyl hydrolases family 17	0.0	12.6	7.9	14.9
beta-1,3-glucanase Glycosyl hydrolases family 17	5.0	0.0	6.1	0.0
endo-1,3-beta-glucosidase	0.0	6.6	0.0	4.3
Glycoside hydrolase family 10 protein (Xylanase)	0.0	5.3	0.0	7.1
Glycoside hydrolase family 10 protein (Xylanase)	0.0	0.0	11.7	0.0
Glycosyl hydrolase family 3 protein (Xylosidase)	6.1	7.5	7.0	5.8
Alpha-L-arabinofuranosidase	2.8	6.5	7.2	7.5
Alpha-L-arabinofuranosidase	5.2	6.9	6.8	6.8
Alpha-L-arabinofuranosidase	3.1	6.2	7.0	7.5
Xyloglucan endotransglycosylase/hydrolase (XTH)	0.0	6.9	0.0	5.3
Xyloglucan endotransglycosylase/hydrolase (XTH)	4.1	4.1	4.2	0.0
Alpha-galactosidase	2.6	0.0	4.8	0.0
Alpha-galactosidase	0.0	13.1	7.9	0.0
MACROFIBRILAR STRUCTURE-RELATED ENZYMES				**17**
Expansin	0.0	5.1	6.4	0.0
Expansin	4.4	5.3	5.8	6.3
Expansin	0.0	6.8	6.8	7.6
Expansin	13.6	0.0	15.1	7.1
endo-1,4-beta-glucanase Glycosyl hydrolase family 9	0.0	9.0	9.2	10.4
endo-1,4-beta-glucanase Glycosyl hydrolase family 9	0.0	0.0	6.7	0.0
endo-1,4-beta-glucanase Glycosyl hydrolase family 9	6.6	8.2	8.1	6.9
Glycosyl hydrolase family 1	2.8	5.8	6.7	6.1
Glycosyl hydrolase family 1	0.0	4.2	6.1	0.0
Glycosyl hydrolase family 1	3.3	6.2	6.7	6.1
Glycosyl hydrolase family 1	0.0	0.0	11.5	0.0
Glycosyl hydrolase family 1	0.0	0.0	7.9	0.0
Glycosyl hydrolase family 1	0.0	0.0	4.9	0.0
glycosyl hydrolase family 3 protein	0.0	0.0	17.3	0.0
glycosyl hydrolase family 3 protein	5.0	5.3	6.3	6.2
glycosyl hydrolase family 3 protein	0.0	7.7	9.1	9.3
				16
TOTAL OF GENES				**48**



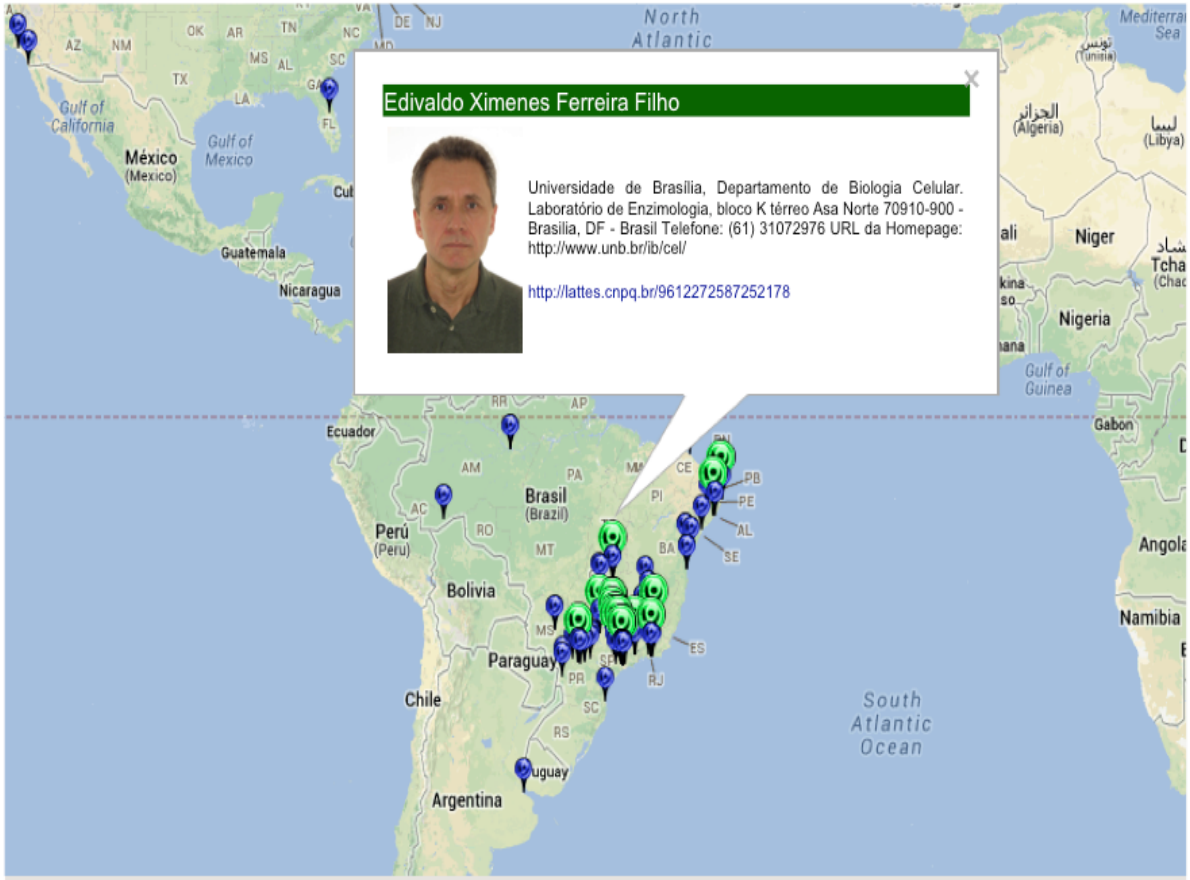
Tavares E.Q.P., De Souza, A.P., Buckeridge, M.S. (2015) How endogenous plant cell wall degradation mechanisms can help achieve higher efficiency in saccahrrification of biomass. Journal of Experimental Botany, doi:10.1093/jxb/erv171

http://www.inctdobioetanol.com.br

Grafo de colaborações



Mapa de geolocalização



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CAPES

BR PETROBRAS

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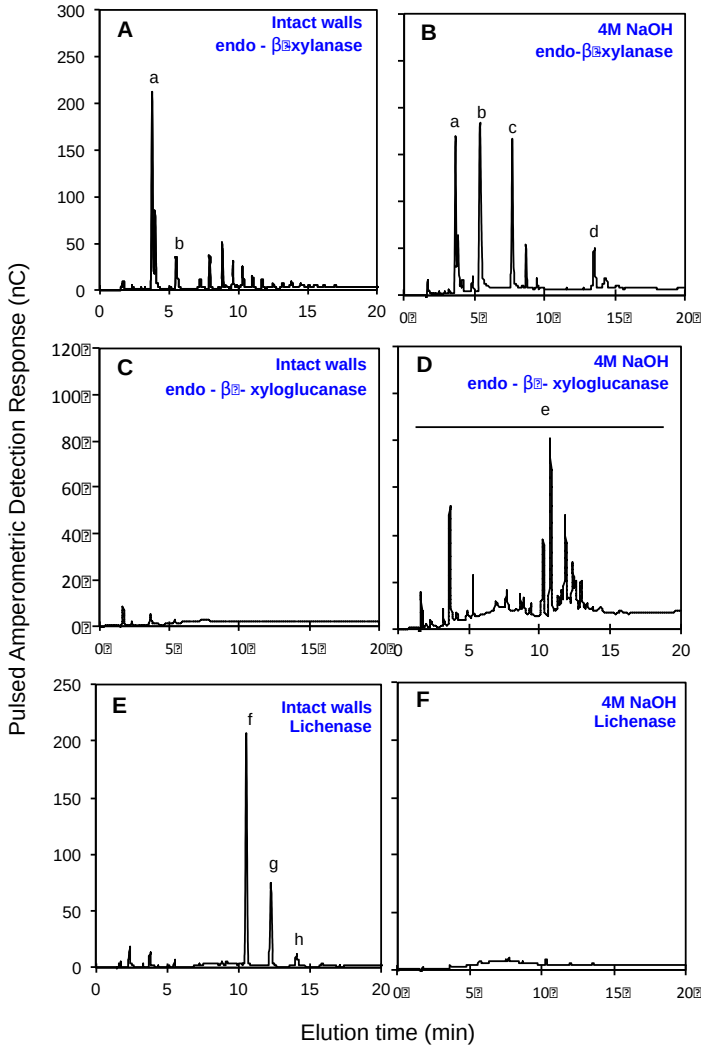
THANK YOU

msbuck@usp.br

Enzyme	organism	Lattice parameters (Angstrons) a	b	c	Reference
Beta-glucosidase	maize	60	118	70	Czjzek et al. Biochem. J. (2001) 354, 37-46
CBH1	Trichoderma reseei	60	**50**	**40**	Divne et al. (1994) Science 265:524-527
Endopolygalacturonase	Aspergillus niger	65,5	201,24	**49,07**	Santen et al. (1999) JBC 274:30474-30480
Pectin Methyl Esterase	carrot	**49,5**	77,6	89,2	Johansson et al. (2002) FEBS Letters 514:243-249
alpha galactosidase	rice	63,7	71,4	84,2	Fujimoto et al. (2003) JBC, 278:20313-20318.
beta-galactosidase	Trichoderma reseei	67,4	69,2	81,5	Maksimainen et al. (2011) J. Str. Biol. 174:156-163
XTH	Nasturtium	116,1	116,1	63,1	Bauman et al. (2007) The Plant Cell 19:1947-<963
Lichenase	barley	**49.6**	82.9	77.5	Muller et al. (1998) JBC 273:3438-3446
beta expansin	maize	**35**	**30**	**24**	Yennawar et al. (2005) PNAS 103: 14664-14671

According to Carpita et al. (Science, 1979, 205:1979-1147) the size exclusion limit for root hair cells of *Raphanus sativus* and *Gossypium hirsutum* are **35-38 and 38-40** Angstrons respectively

For sugarcane stalks, Maziero et al. (J.Agr.Food Chem, 2013) calculated **50 Angstrons**, varying from more to less porous from top to bottom of the plant



TWO EVIDENCES FOR THE ROLE OF POROSITY IN RECALCITRANCE

Buckeridge, M.S., Dos Santos, W.D., Tiné, M.A.S., De Souza, A.P. (2015) The Cell Wall Architecture of Sugarcane and its Implications to Cell Wall Recalcitrance. Compendium of Bioenergy Crops: Sugarcane edited by Eric Lam. CRC Press, Taylor and Francis