LABORATORY OF MICROBIOLOGY AND CELLULAR BIOLOGY

Prospecting Hydrolases of microorganisms and finding their potential for bioenergy production

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Biology Department

Presentation outline

Biomass

Biofuels

Enzymes from microorganisms

Perspectives

Biomass

What is it?

All renewable resources which come from organic matter and that can be used to produce energy.

Three domains of life and their wastes

2.000.000.000.000 tons

Source: Fair Biomass Brazil

Biofuels

Solid, liquid or gaseous fuel

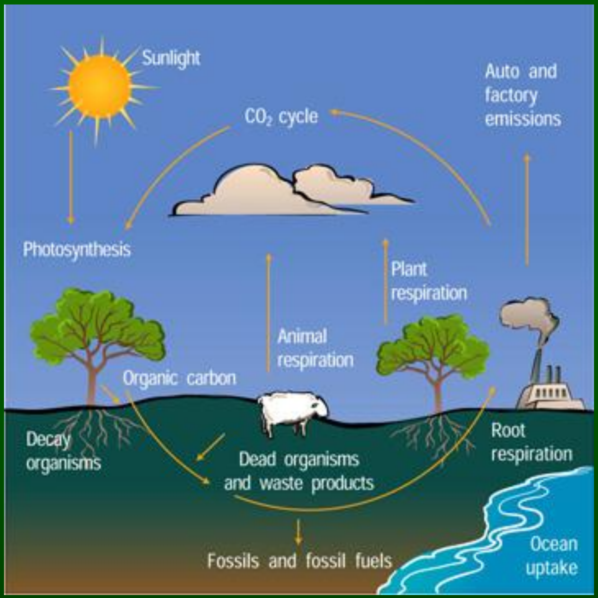
wood, grass cuttings, domestic refuse, charcoal, dried manure, residues

Vegetable oil, alcohol, biodisel

Syngas, Biogas

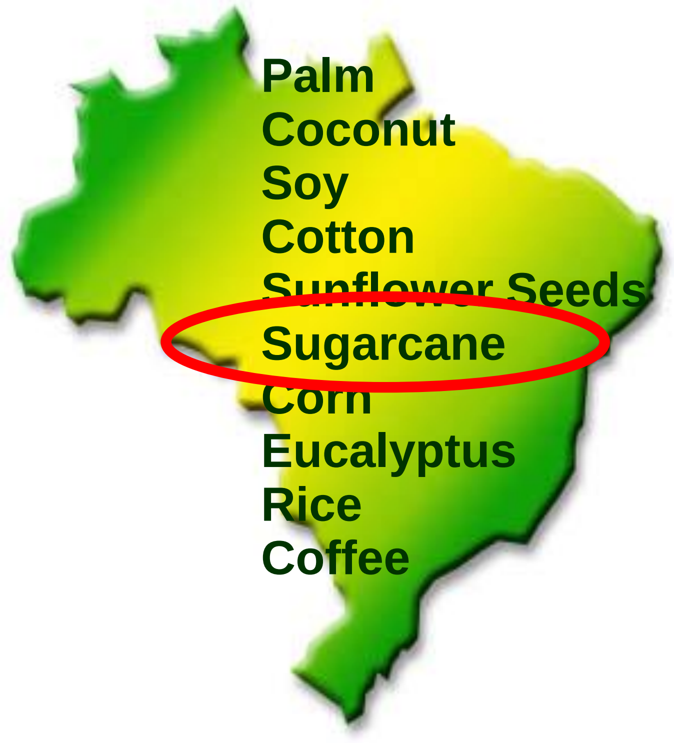
Advantages

Biofuels

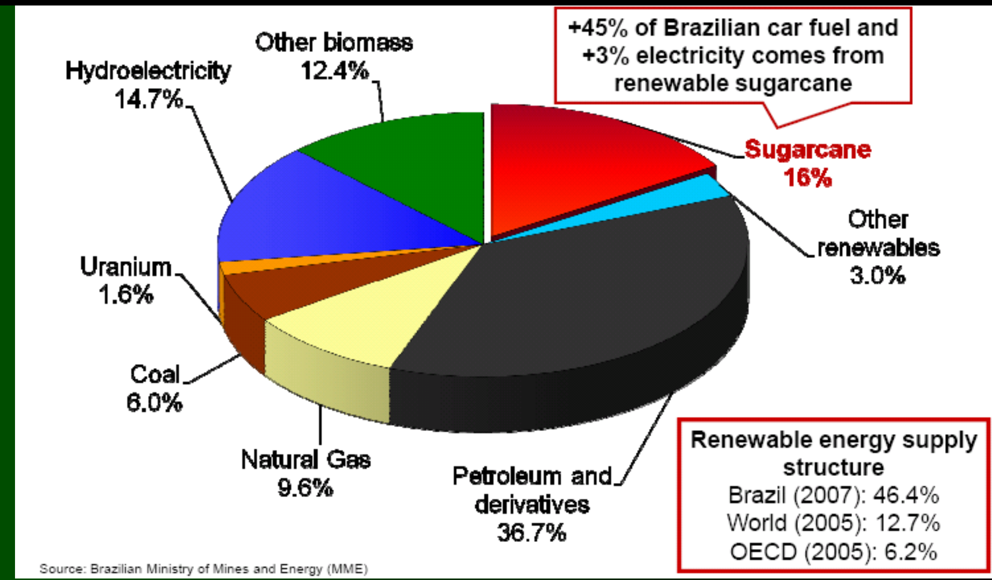


ENERGY

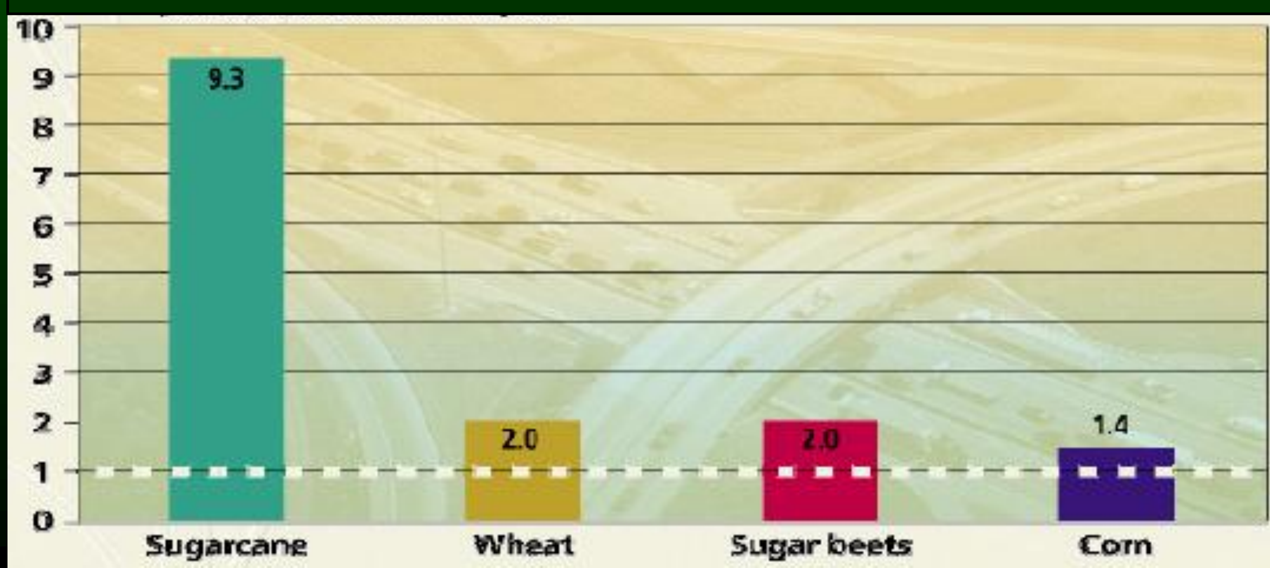
GONE



Brazil Energy Matrix Input

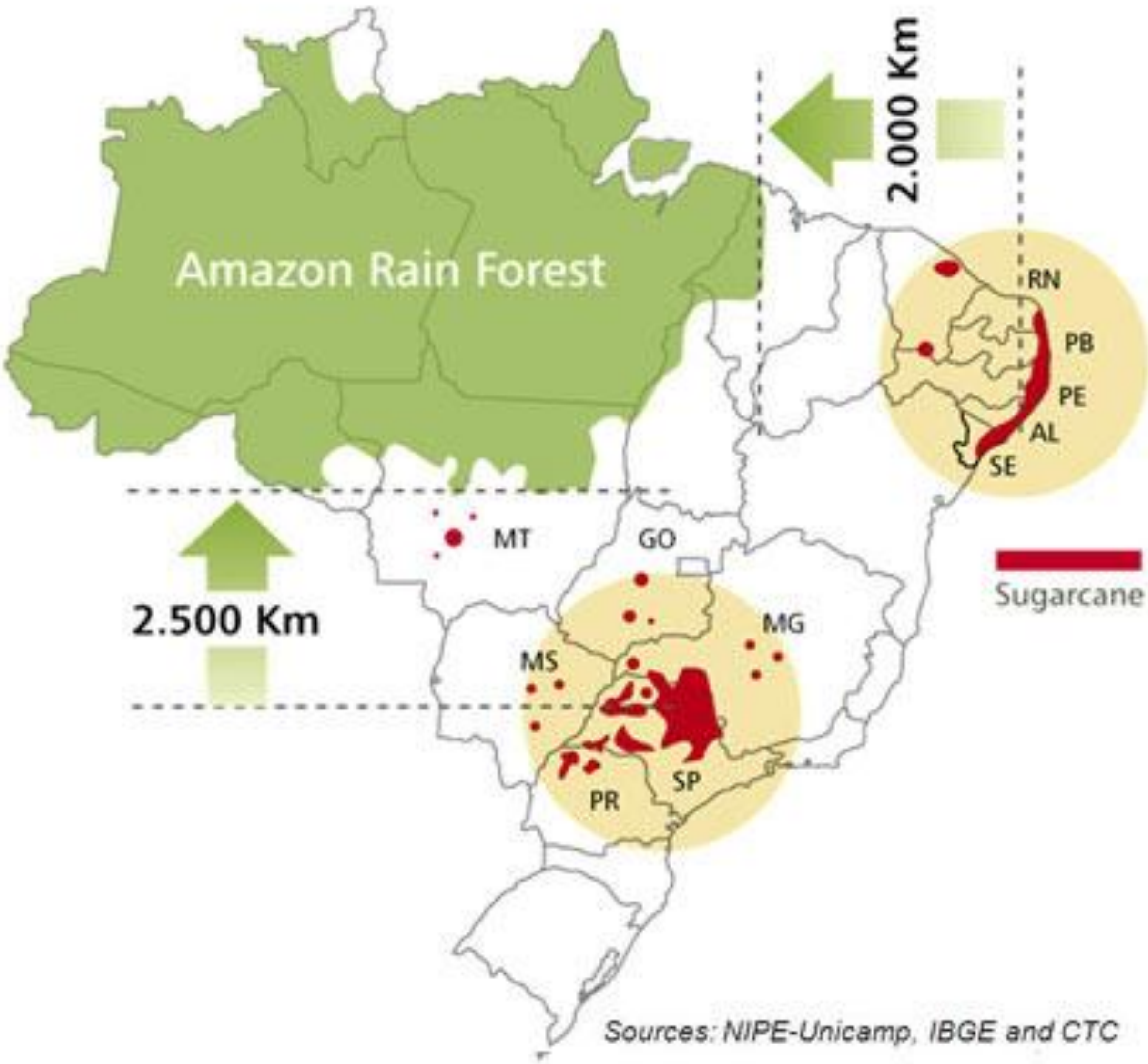


SUPERIOR ENRGY BALANCE



Fonte: World Watch Institute (2005) and Macedo et al. (2008)

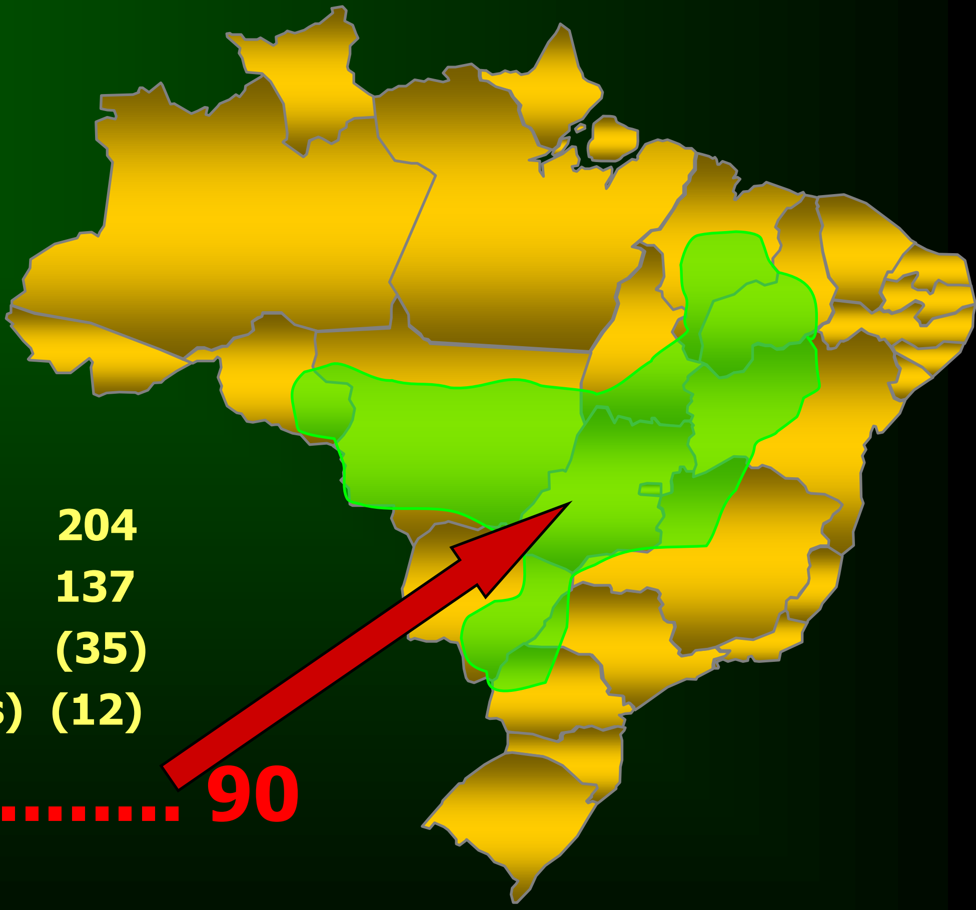
Sugarcane producing regions in Brazil



BRAZIL: EXPANSION POTENTIAL

BRAZIL IS THE ONLY COUNTRY IN THE WORLD WITH GREAT CAPACITY TO EXPAND ITS AGRICULTURAL PRODUCTION (with sustentability)

Expansion area in the “ Brazilian Cerrado” region



(million hectares)

Total Area	204
Area good for agriculture......	137
Area in use for cattle raising...	(35)
Occupied area (forests & plantations)	(12)

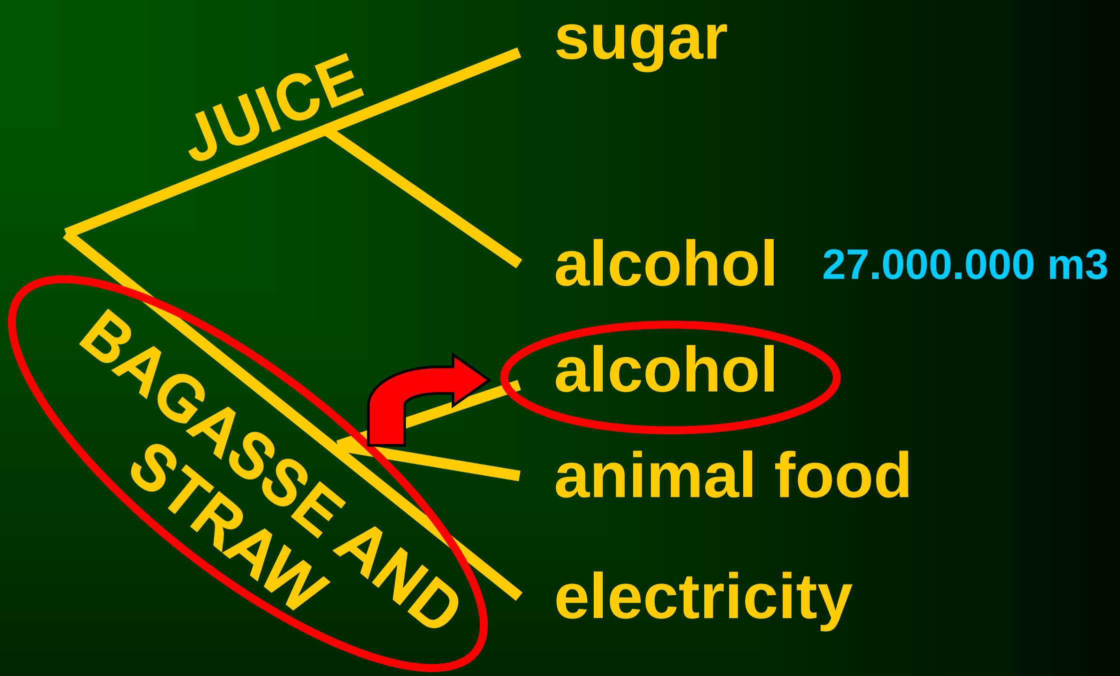
Available Area 90

Source: EMBRAPA

Second generation ethanol

Sugarcane

426 million tons



Fonte: CONAB

Ethanol

It is biodegradable, made from renewable sources

Low toxicity to aquatic and soil forms of life

Virtually no sulfur and particulate emissions

Reduces emissions of CO and toxic substances

Blended with other fuels

Manufacture biodisel (methanol)

Source of hydrogen (fuel cells)

Increasing Market of flexible fuel vehicles

Alternative fuel for motorcycles, boats, airplanes

Plastic production

Electricity

Cellulosic ethanol

600.000.000 tons

75.000.000 tons

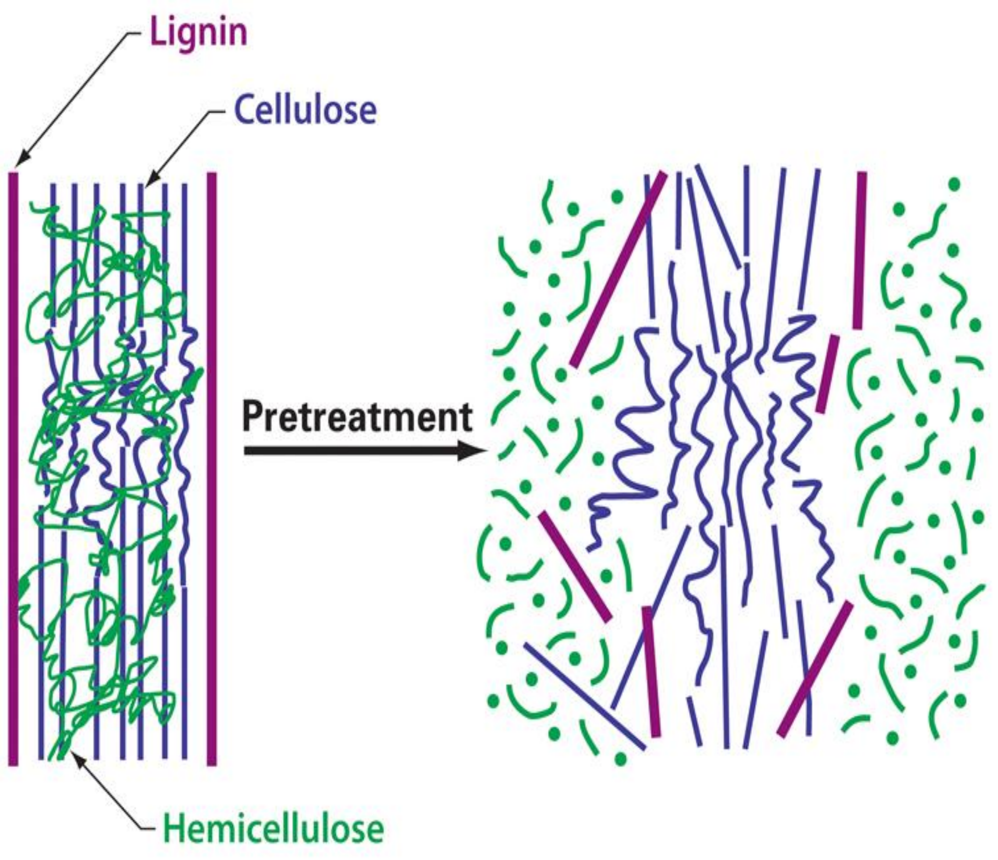
61.200.000 tons

10.000.000 tons

2.400.000.000 L

Fonte: UNICA

Cell wall structure



Polizeli (2008). Current Advances in Fungal Biotechnology.

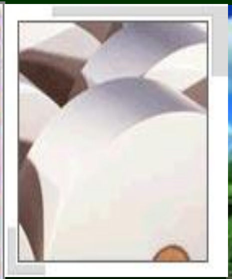
Enzymes:

Amylases	*Aspergillus phoenicis, A. niveus; Rhizopus microsporus var. rhizopodiformis; Paecilomyces variotii*
Celulases	*Aspergillus phoenicis, A. niveus; A. japonicus; A. ochraceus; A. niger; A. terricola; Malbranchea puchella*
Xylanases	*Aspergillus phoenicis, A. niveus; A. japonicus; A. ochraceus; A. niger; A. terricola; Malbranchea puchella*
Xylosidases	*Aspergillus phoenicis, A. niveus; A. japonicus; A. ochraceus; A. niger; A. terricola; Malbranchea puchella*
Ligninases	*Aspergillus phoenicis, A. niveus; A. japonicus; A. ochraceus; A. niger; A. terricola; Malbranchea puchella*
Invertases	*Aspergillus phoenicis; A. caespitosus*
Lipases	*Trichoderma pseudokoningii; A. caespitosus; A. niger; Cordyceps brongniartii;Penicillium purpurogenum*
Pectinases	*Aspergillus terreus; A. niveus; Rhizopus microsporus var. rhizopodiformis; Paecilomyces variotii*
Acid and alkaline phosphatases	*Aspergillus niger; A. niveus; A. ochraceus.*
Phytases	*Aspergillus niger; A. niveus; A. ochraceus.*


Syrup starch


Cheese preparation


Fruit juice


Pulp & paper


Animal food


detergent

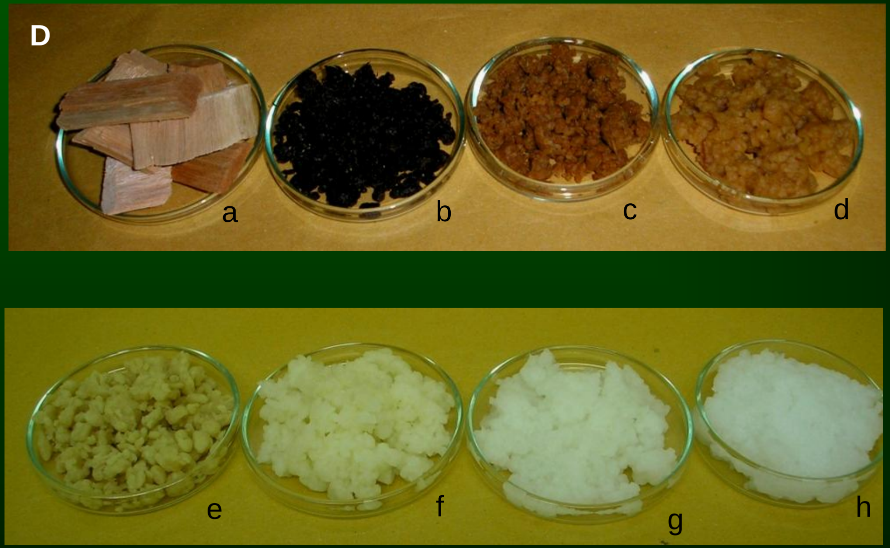
Ethanol

BIOBLEACHING OF CELLULOSIC PULP

A

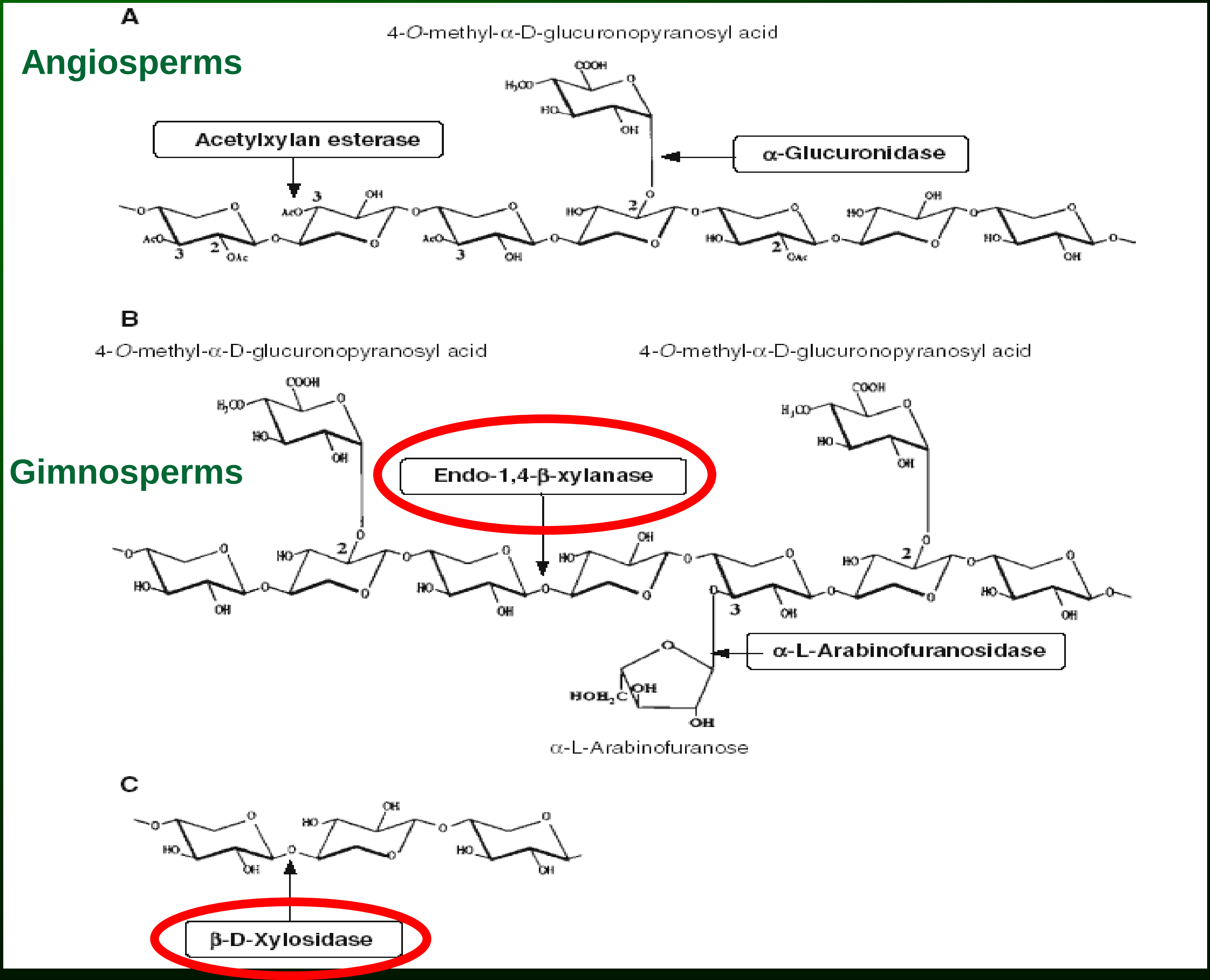
B

C

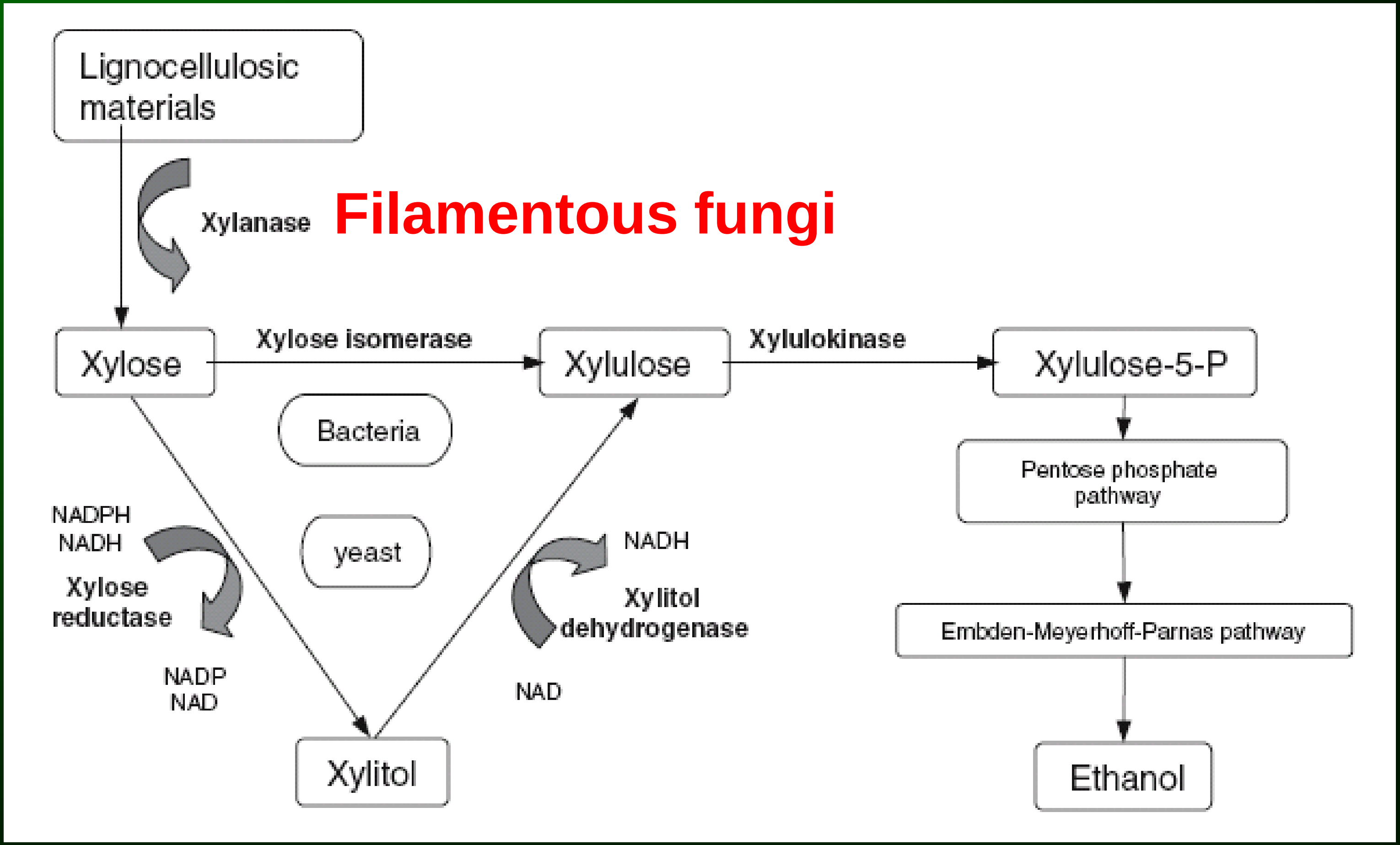


(f) $NaOH/O_3/ClO_2$ (g) $NaOH/O_2/H_2O_2$ (h) $NaOH/ClO_2$

Polizeli, M.L.T.M. Properties and Commercial Applications of Xylanases from fungi. *In: Mycotechnology: Current Trends and Future Prospects*, I.K. Int. Publisher, 4: 82-108, 2008.



Polizeli, M.L.T.M. et al., Xylanases from fungi: properties and industrial applications. Review. *Appl. Microbiol. Biotechnol.*, 67, 577-591, 2005



Polizeli, M.L.T.M. et al., Xylanases from fungi: properties and industrial applications. Review. *Appl. Microbiol. Biotechnol.*, 67, 577-591, 2005

Collection of samples

beehive

Soil and leaves

Sugar cane soil

Soil and humus of farm

edge of the river

Thermal water

Orange trees soil

Eucalipto forest

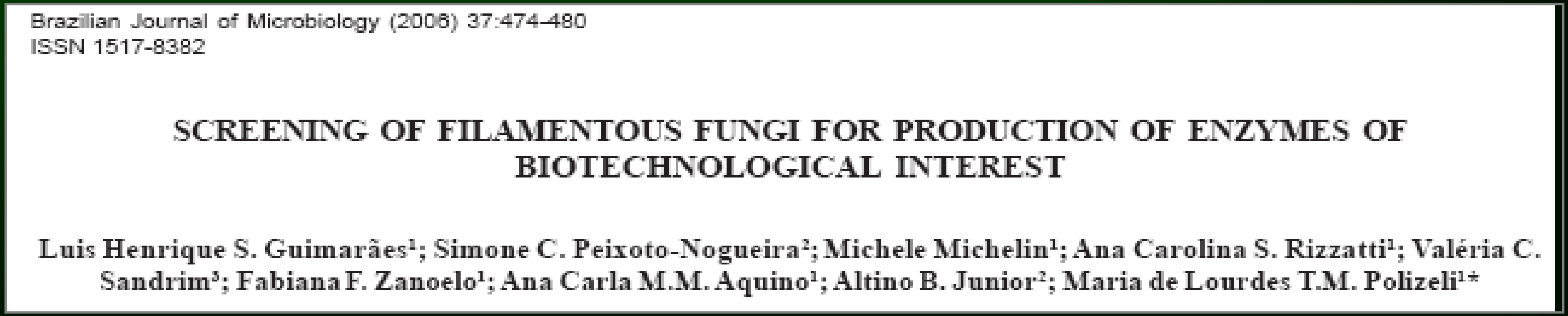
Maize plantation

Fungus samples

Fungus isolates

Fungi storaging

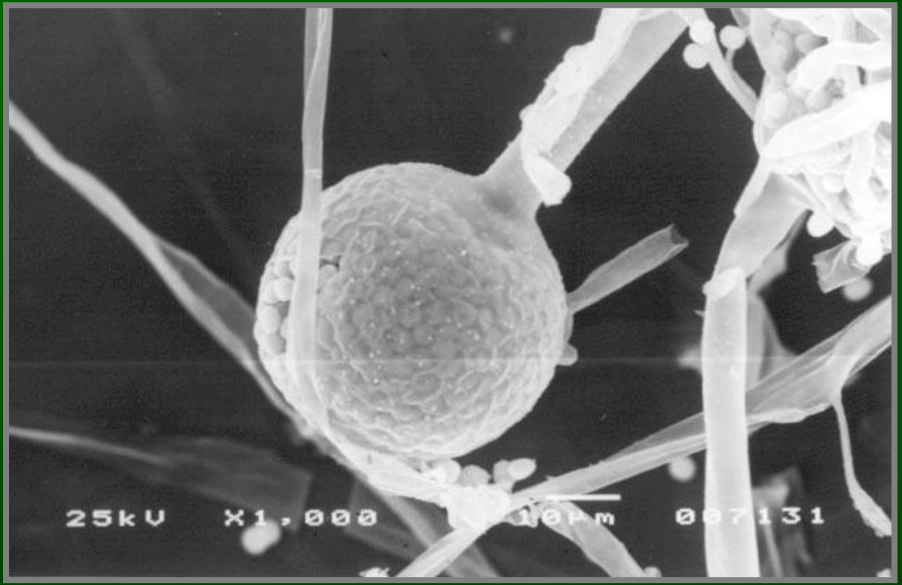
Fungi characterization

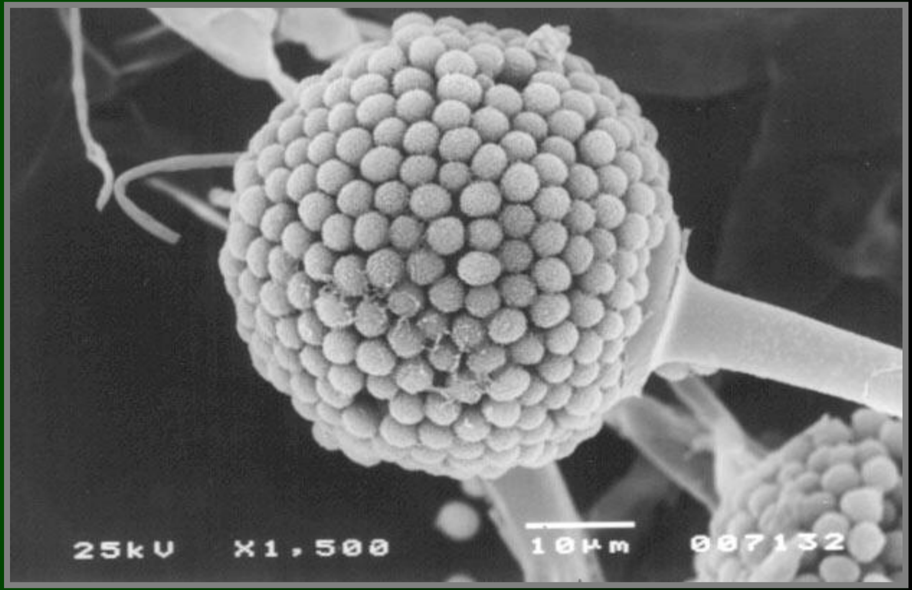

Brazilian Journal of Microbiology (2006) 37:474-480
ISSN 1517-8382

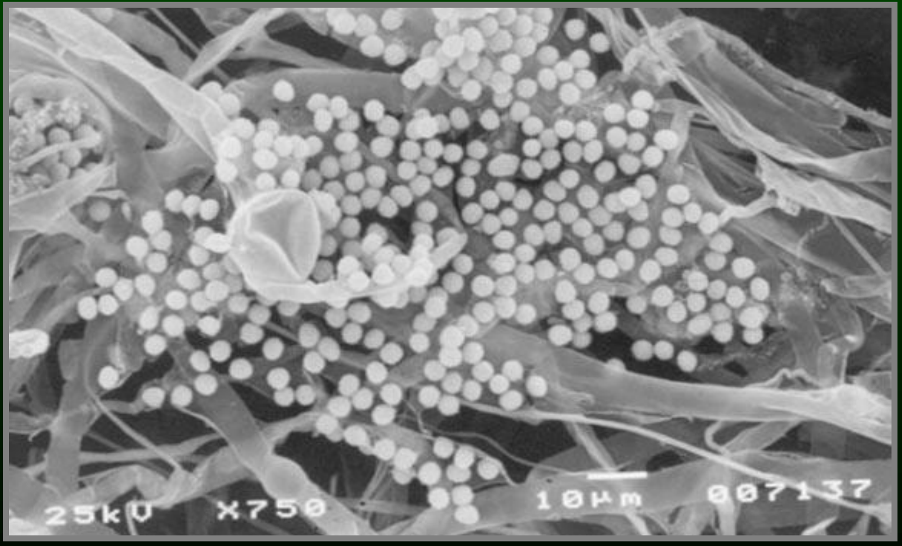
SCREENING OF FILAMENTOUS FUNGI FOR PRODUCTION OF ENZYMES OF BIOTECHNOLOGICAL INTEREST

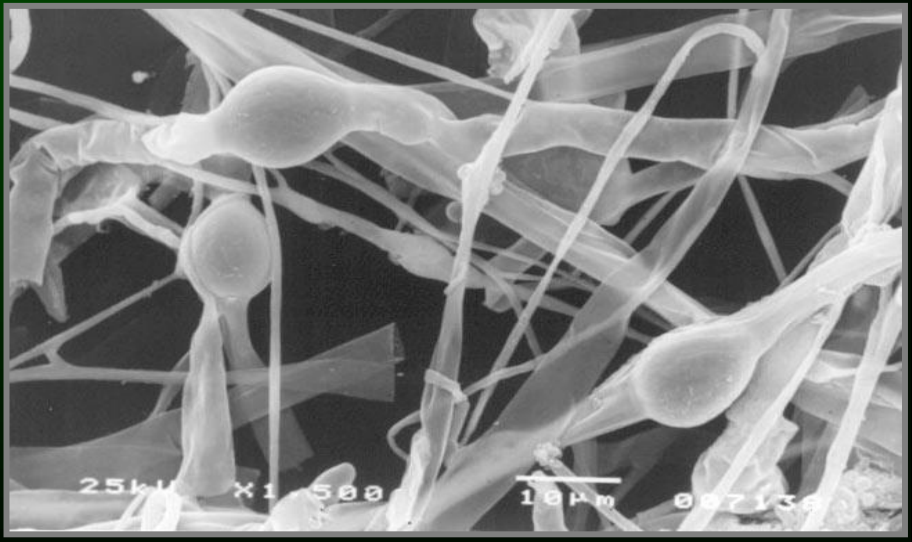
Luis Henrique S. Guimarães[1]; Simone C. Peixoto-Nogueira[2]; Michele Michelin[1]; Ana Carolina S. Rizzatti[1]; Valéria C. Sandrim[3]; Fabiana F. Zanoelo[1]; Ana Carla M.M. Aquino[1]; Altino B. Junior[2]; Maria de Lourdes T.M. Polizeli[1]*

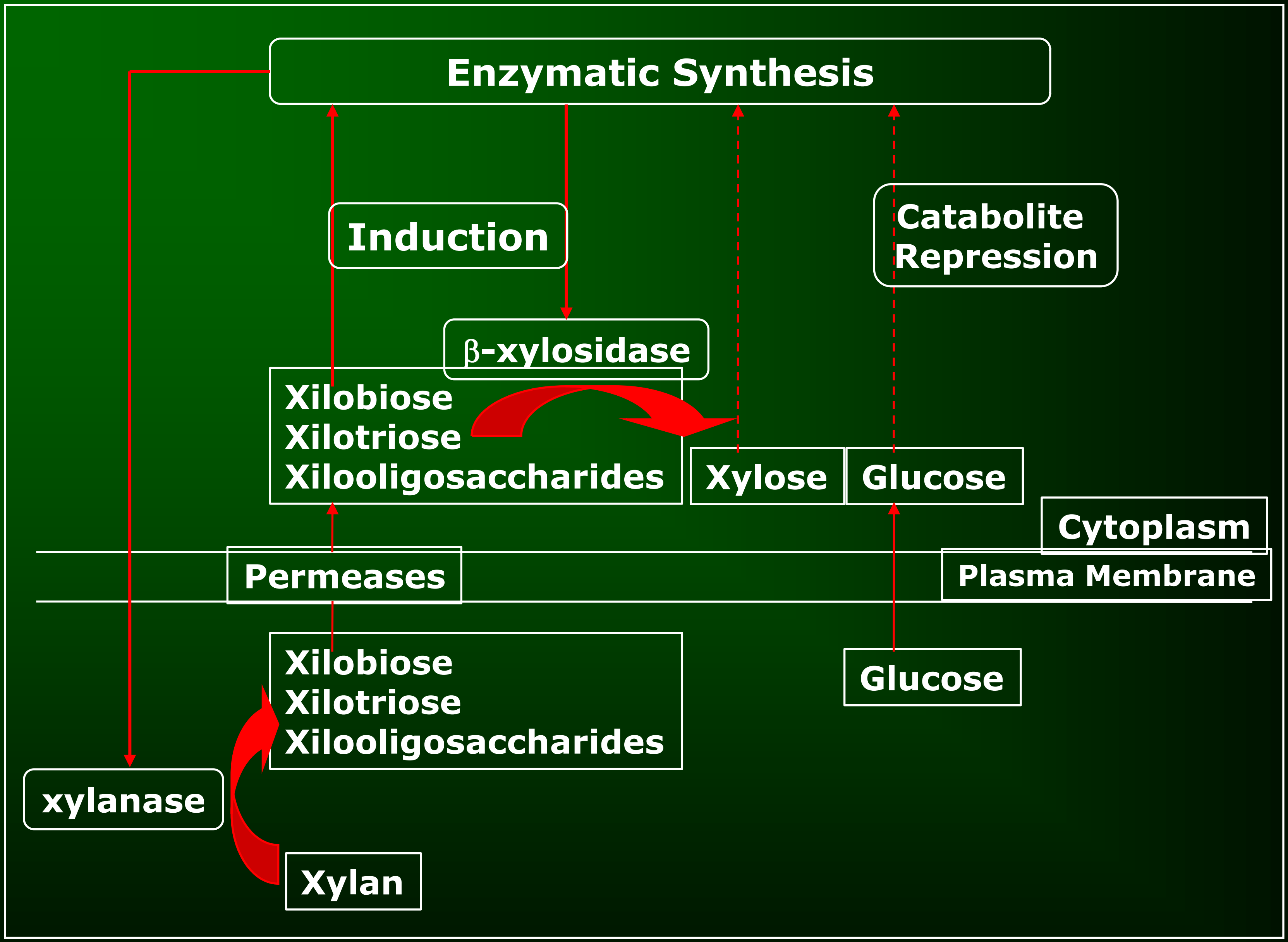
Fungi characterization











Polizeli, M.L.T.M. et al., Xylanases from fungi: properties and industrial application. *In:* Appl Microbiol Biotechnol 67: 577-591, 2005.

Effect of carbon sources on ß-xylosidase and xylanase production

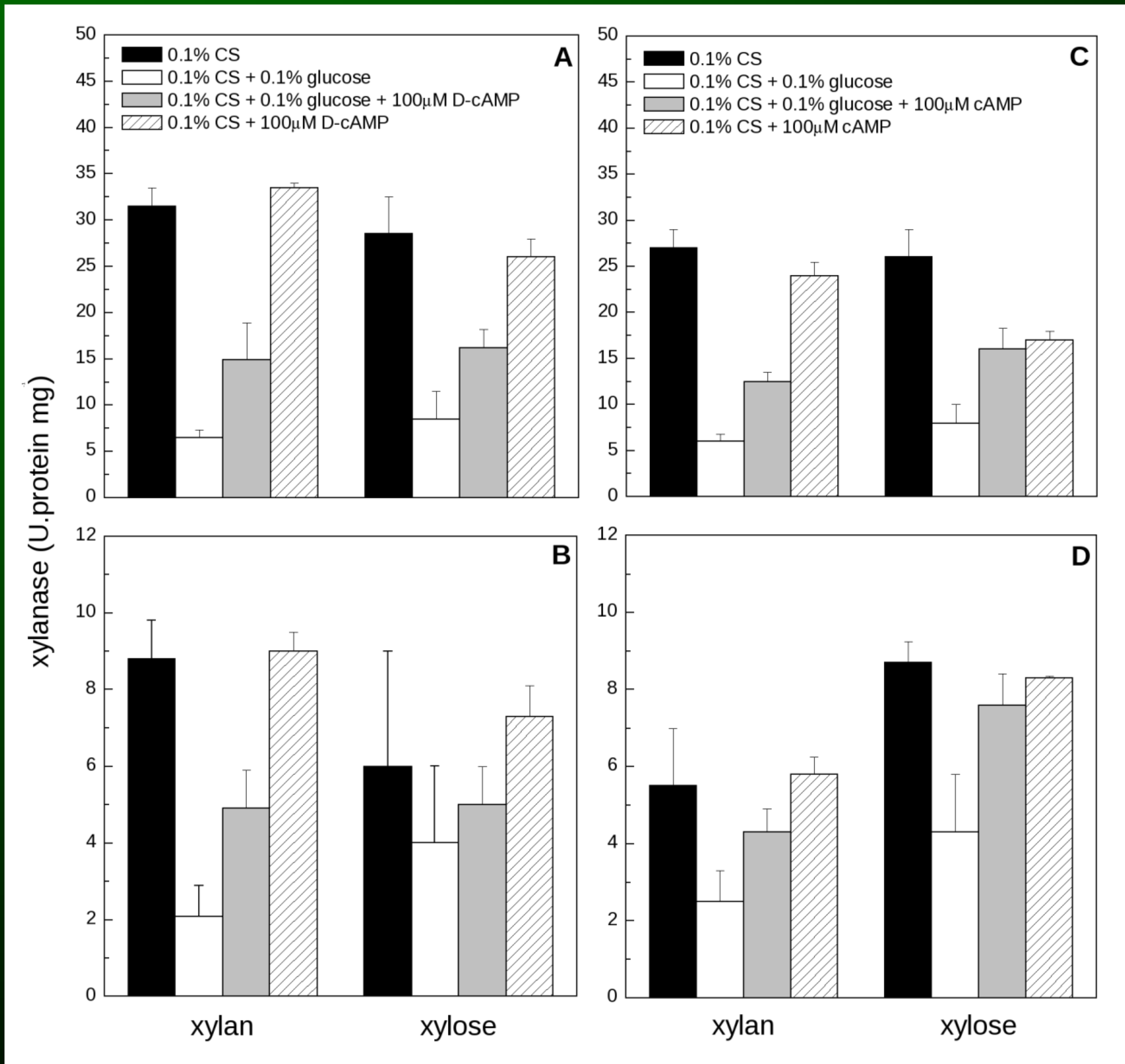
Carbon source	Concentration (%)	Protein (mg total)	Specific activity, U (mg protein)$^{-1}$
None		1.5	8.6
Sugars			
Avicel	1	1.7	nd[b]
Cellobiose	2	1.8	95.4
Fructose	2	3.6	45.7
Glucose	2	4.7	34.5
Maltose	2	4.4	43.4
Raffinose	2	1.3	nd
Starch	1	1.6	82.9
Sucrose	2	1.9	nd
Xylan	1	2.3	278.3
Xylose	1	1.8	146.7
Industrial residues			
Bagasse sugar cane	1	2.9	219.9
Cassava flour	1	3.3	nd
Maize pith	1	4.0	112.3
Oatmeal	1	3.1	nd
Rice peal	1	2.7	nd
Wheat raw	1	5.2	35.0

Polizeli, M.L.T.M. et al., *J. Ind. Microb. & Biotechnol.* 26, 156-160, 2001.

Carbon source (1%)	Extracellular xylanase (U mg protein^{-1})
Control[a]	1.15
Arabinose	8.90
Fructose	3.31
Galactose	1.70
Glucose	2.37
Mannose	2.50
Ribose	2.22
Xylose	152.00
Xylose + glucose	40.00
Cellobiose	1.52
Raffinose	1.42
Lactose	0
Maltose	5.90
Sucrose	4.14
Starch	13.10
Xylan (birchwood)	172.40
Xylan (oat spelt)	171.40
Xylan (beechwood)	173.00
Xylan + glucose	90.00
Xylitol	3.14
β-Methylxyloside	152.30
β-Methylxyloside + glucose	25.00

Rizzatti, A. C. et al., J. Ind. Microbiol. Biotechnol. 35 (4), 237- 244,.2008.

Effect of glucose, dibutyryl-cAMP or cAMP on xylanase production



Extracellular activity

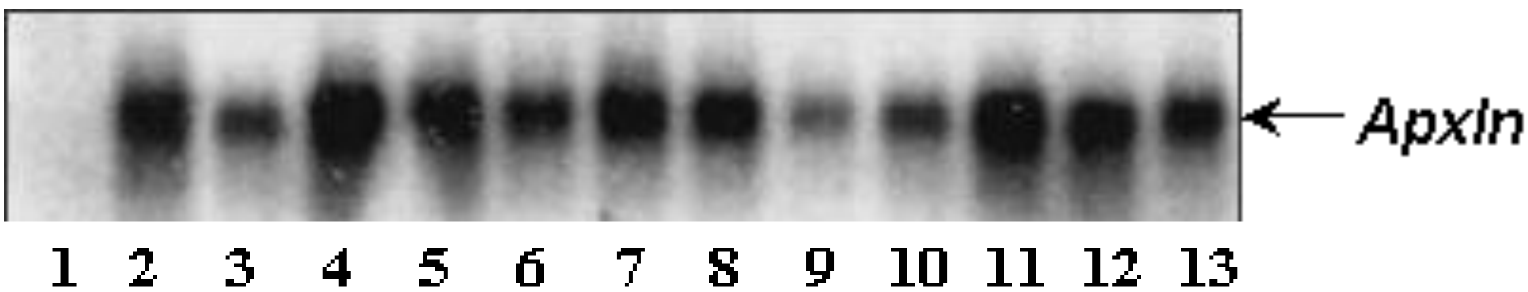
Mycelial extract

The fungus was previously grown on Vogel medium supplemented with 1% glucose, during 72 h, at 42 C and transferred to the different media during 6 h. *N* = 4.

Polizeli, M.L.T.M. *J. Ind. Microbiol. & Biotechnol.* 35: 237-244, 2008.

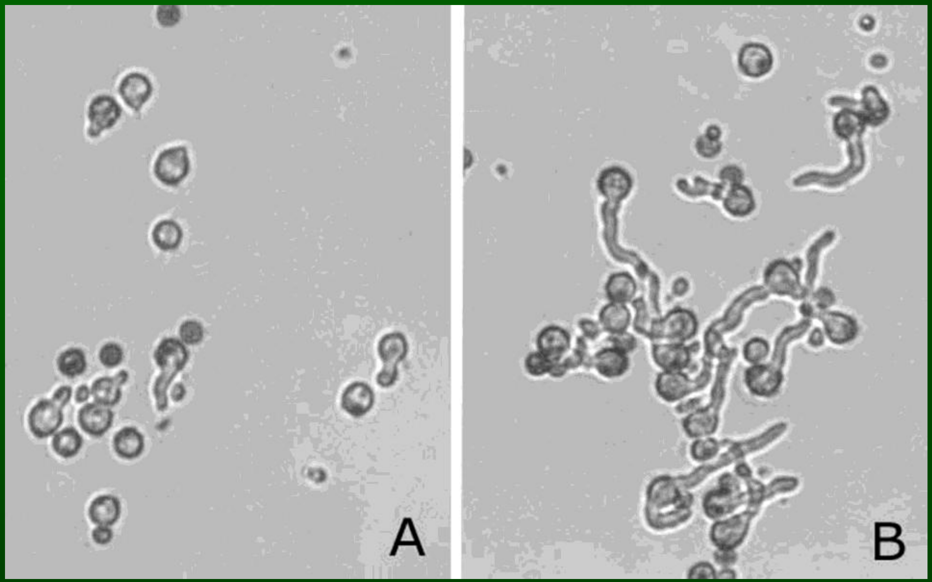
Northern blot hybridization of the *A. phoenicis* gene *ApXLN*

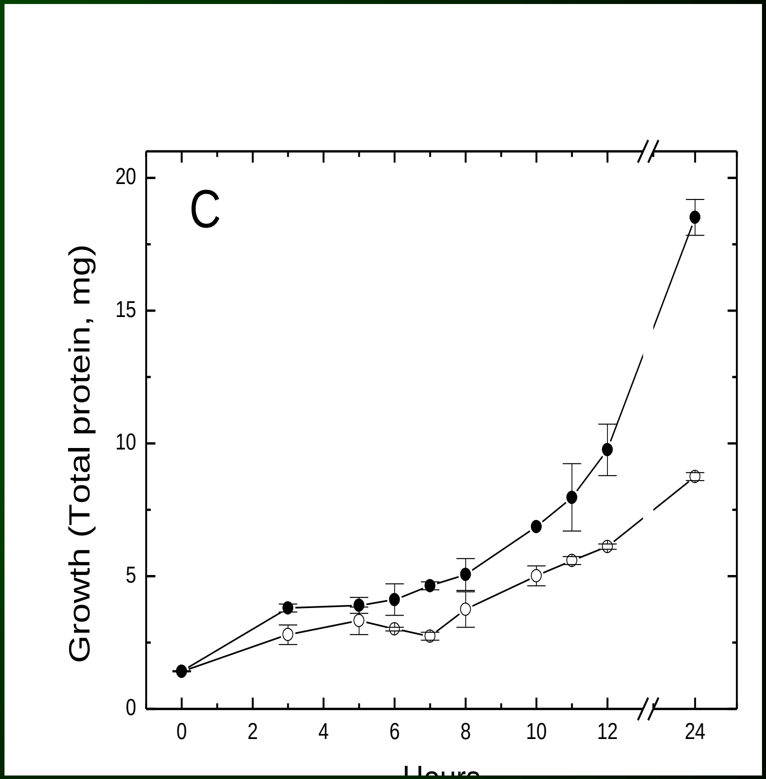
	1	2	3	4	5	6	7	8	9	10	11	12	13
0.1% xylan	−	+	+	+	+	+	+	−	−	−	−	−	−
0.1% glucose	−	−	+	+	−	+	−	−	+	+	−	−	+
0.1% xylose	−	−	−	−	−	−	−	+	+	+	+	+	+
100 µM cAMP	−	−	−	+	+	−	−	−	−	+	+	−	−
100 µM d-cAMP	−	−	−	−	−	+	+	−	−	−	−	+	+



Polizeli, M.L.T.M. *J. Ind. Microbiol. & Biotechnol.* 35: 237-244, 2008.

Spore germination of *A. phoenicis* at 25 C (A) and 42 C (B) for 8 hs

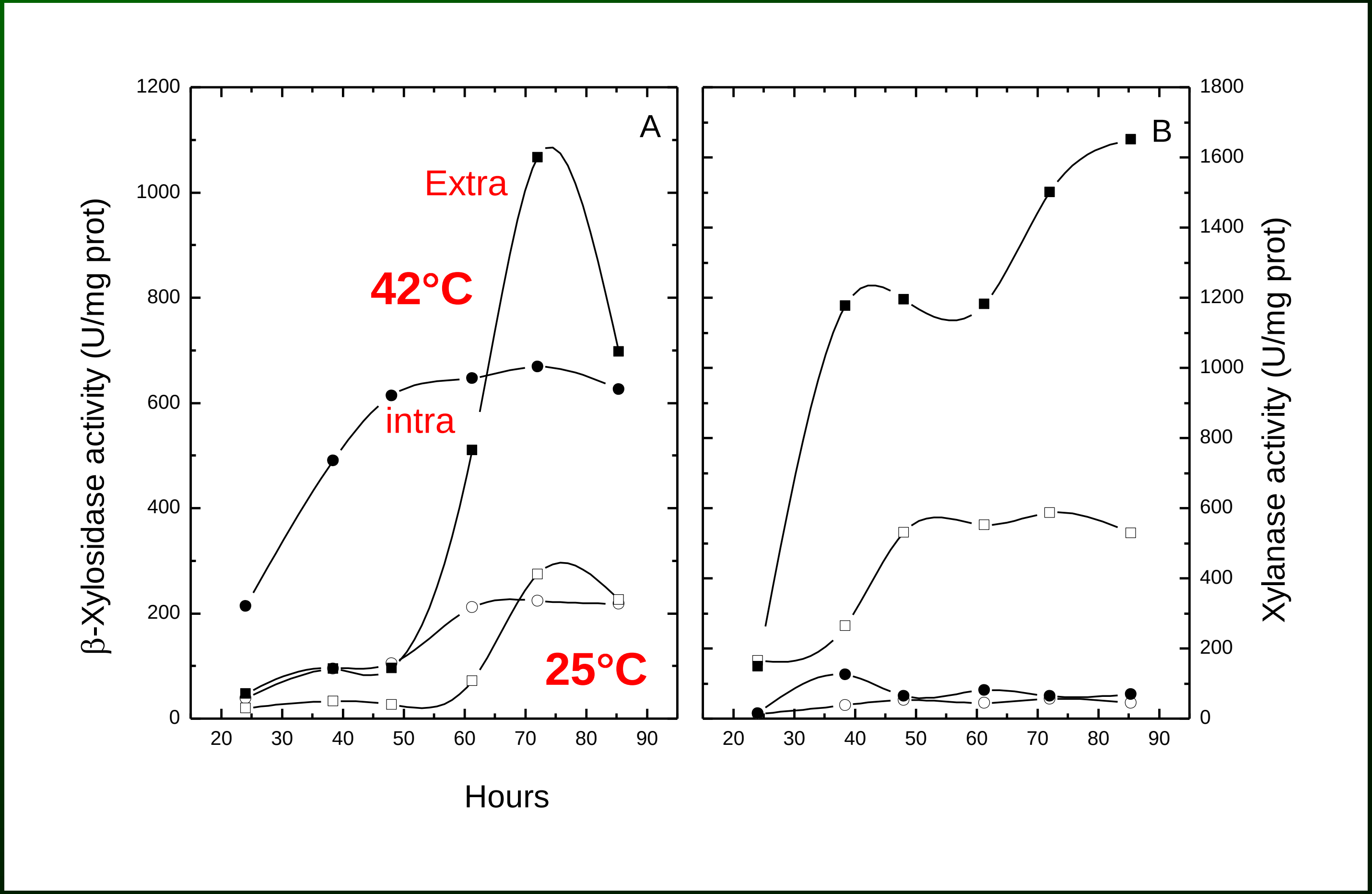




(C) Growth at 25 C (○) and 42 C (●).

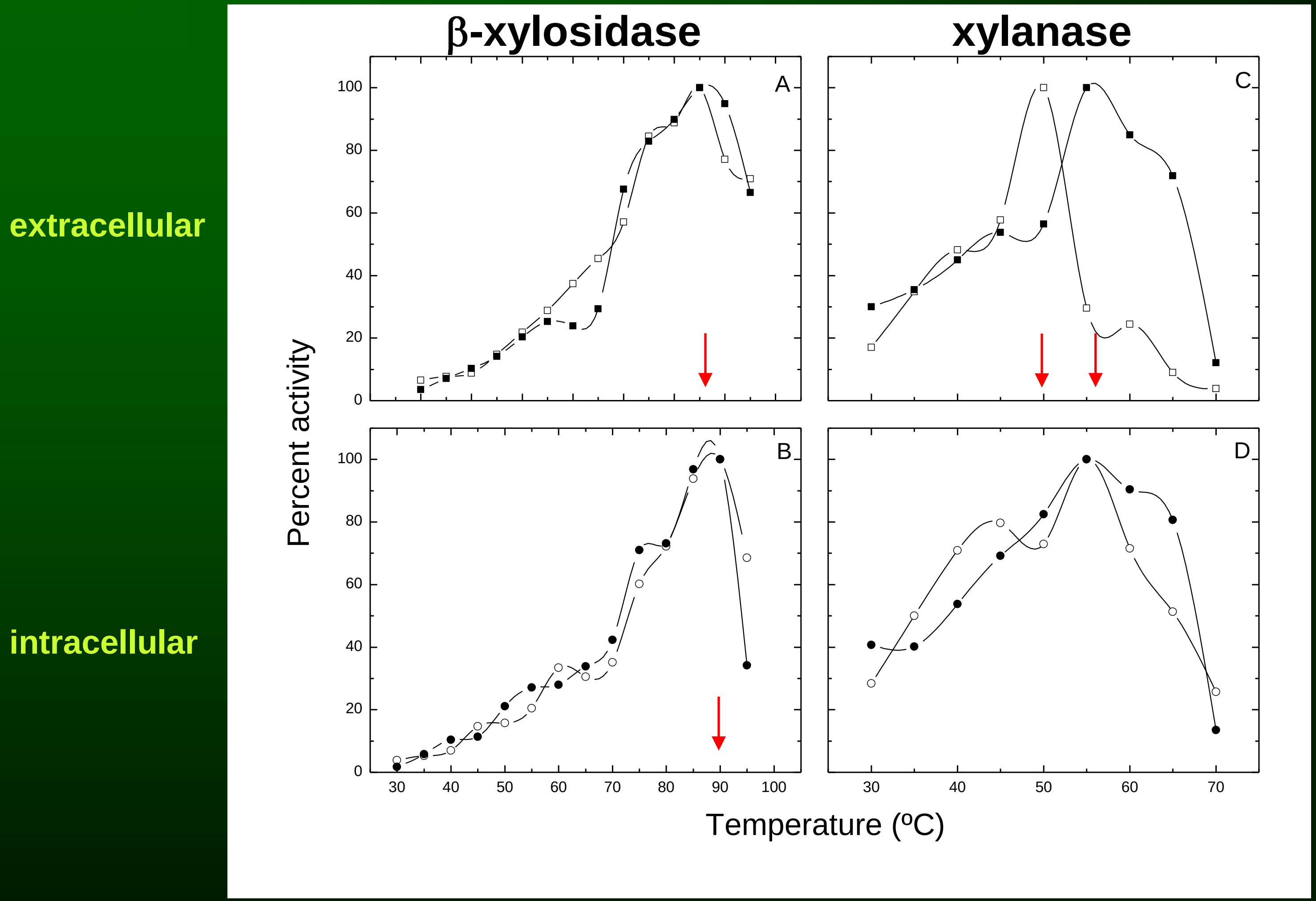
Polizeli, M.L.T.M. et al., *J. Ind. Microbiol. & Biotechnol.* 31, 88-93, 2004.

Time-course of β-xylosidase (A) and xylanase (B) production



Polizeli, M.L.T.M. et al. *J. Ind. Microbiol. & Biotechnol.* 31, 88-93, 2004.

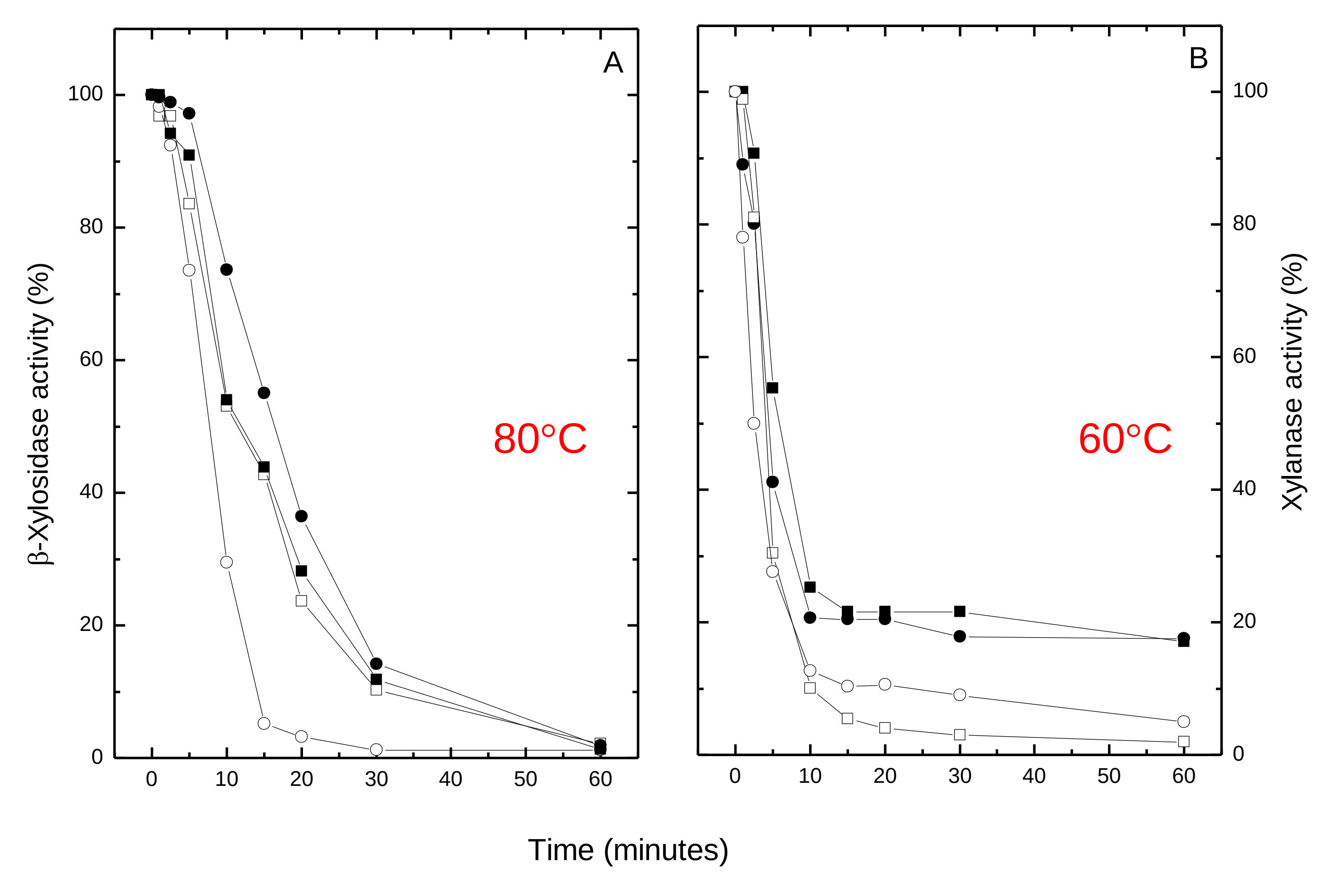
Effect of temperature on activities



25°C (□, ○), 42°C (■, ●)

Polizeli, M.L.T.M. *J. Ind. Microbiol. & Biotechnol.* 31, 88-93, 2004.

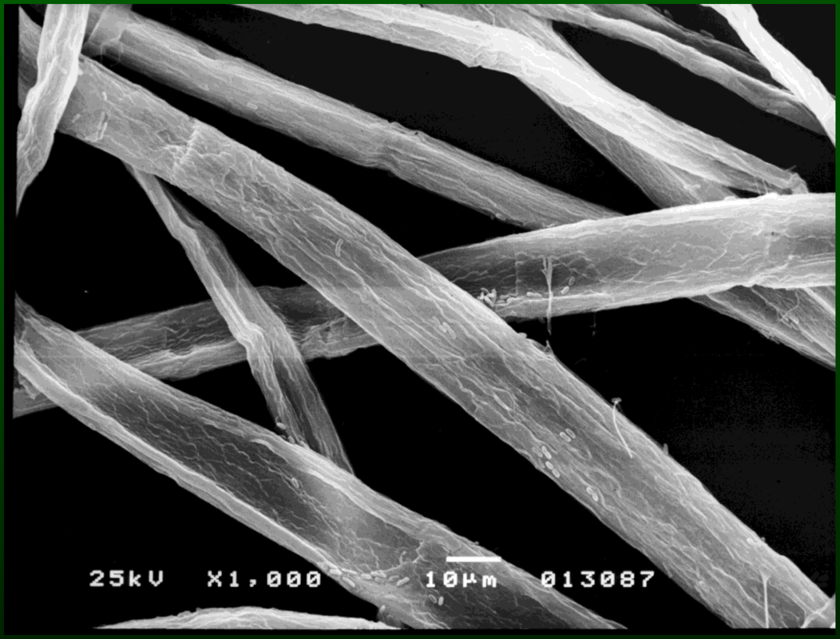
Thermal inactivation



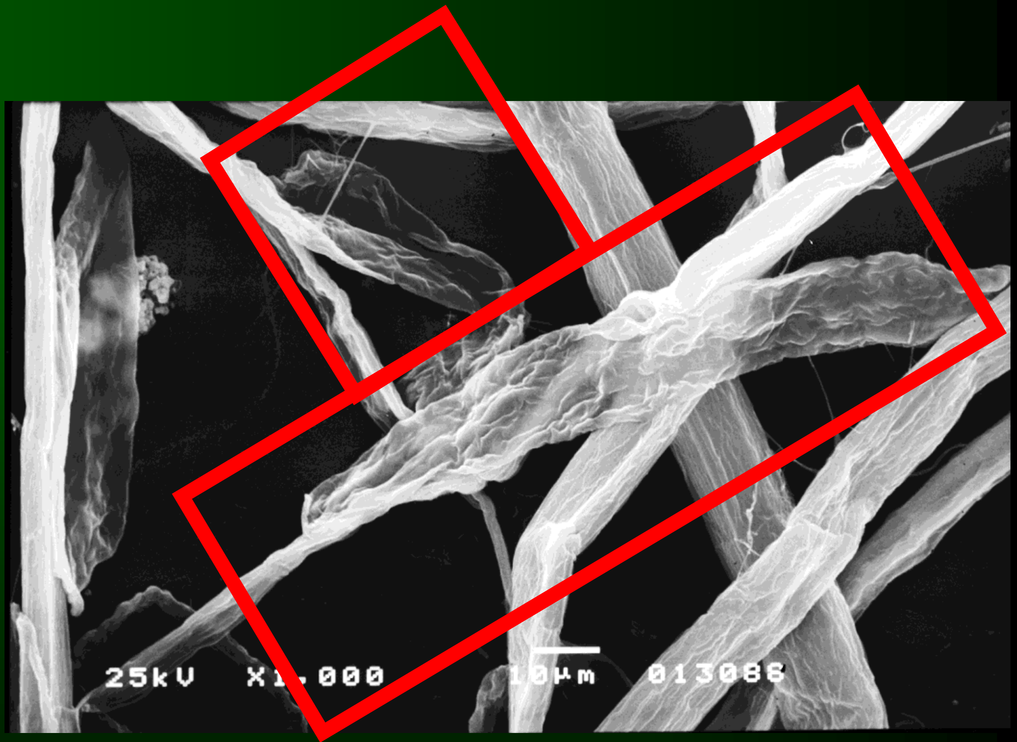
25°C (□, ○); 42°C (■, ●); extra (□■), intra (○●)

Polizeli, M.L.T.M. et al. *J. Ind. Microbiol. & Biotechnol.* 31, 88-93, 2004.

ELECTRONIC MICROSCOPY OF CELLULOSE TREATED WITH XYLANASE



control

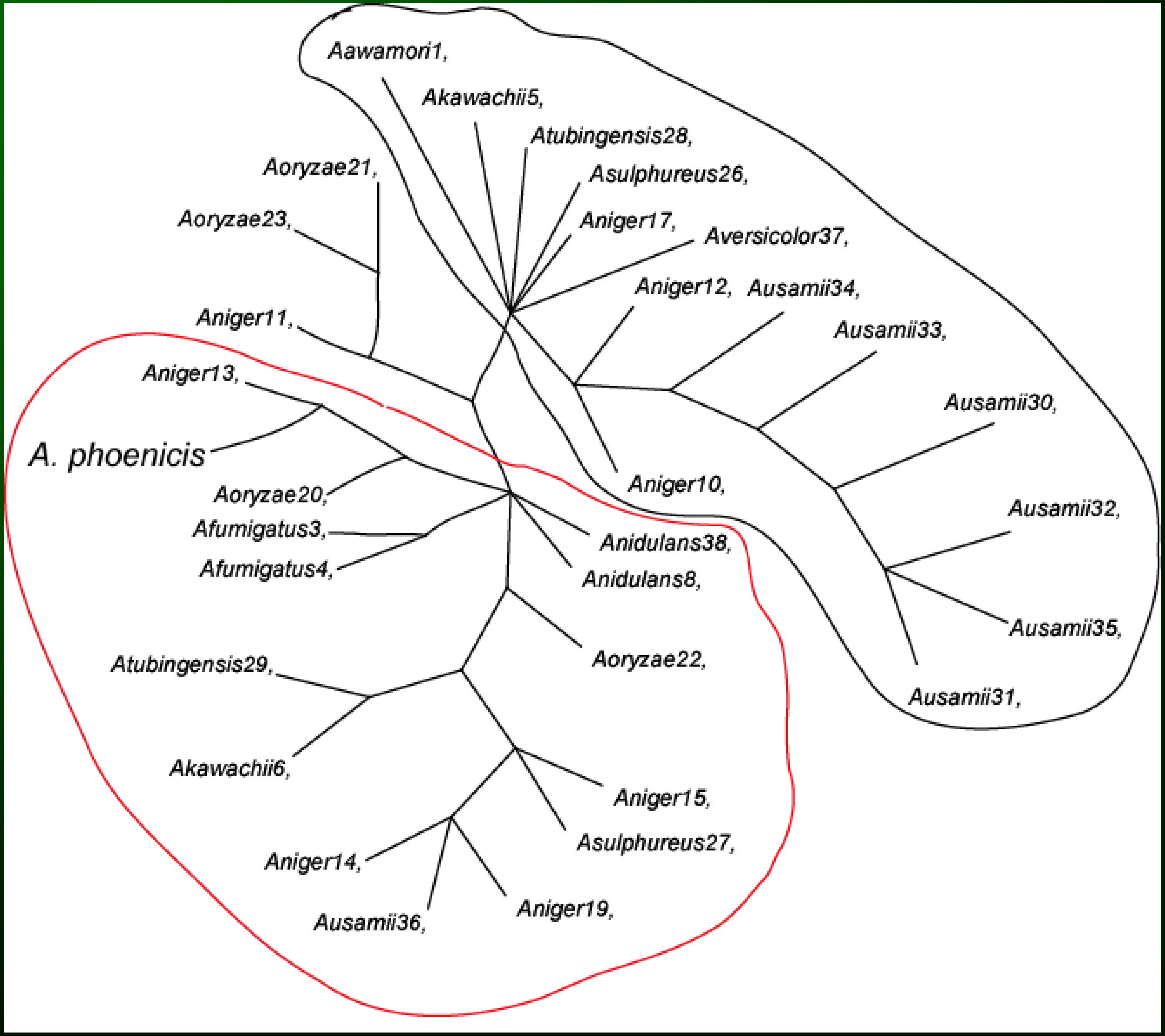


2 hours of incubation Xylanase

15 U/g dry cellulose pulp

Polizeli, M.L.T.M. et al. submitted.

PHYLOGENETIC RELATIONSHIPS AMONG GENUS *Aspergillus*



Corrêa, E. C. et al. (submmited to Molecular Biology and evolution) 2008.

Perspectives

Development of Enzymes for Biomass Saccharification

OUR LABORATORY

Overexpression of enzymes in heterologous organisms

Production of enzymes and fungi quick adaptation (directed anagenetic evolution)

Comparative and evolutionary approaches of different groups of enzymes

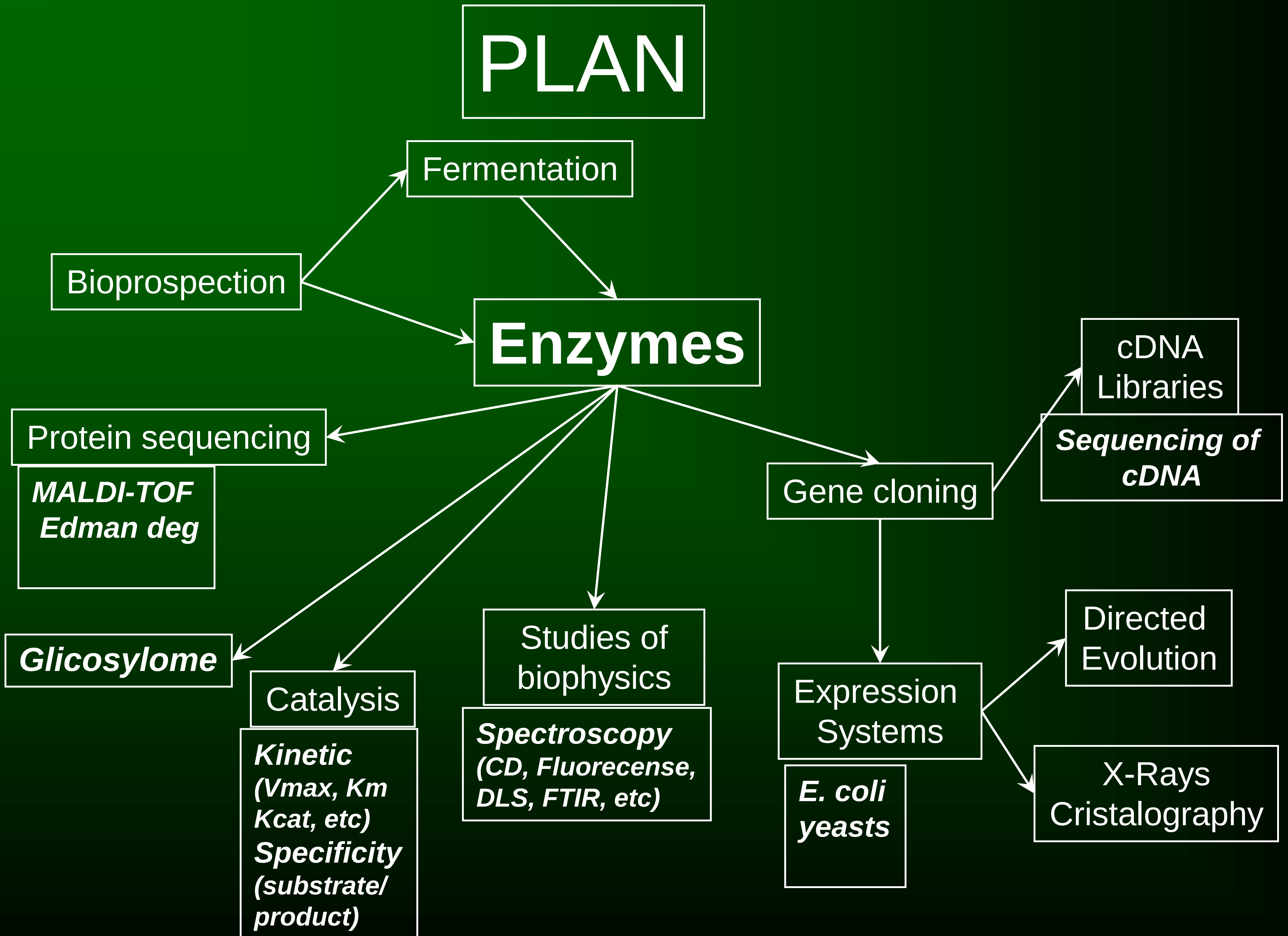
purification and biochemical characterization of microbial enzymes and sequencing

catabolic induction and repression studies using diverse carbon sources

Submerged and solid-substrate fermentations (using residues from agro-farming and forestry industries)

Sparse matrix techniques for optimization of microbial enzyme production

Bioprospection of filamentous fungi


PLAN
Fermentation
Bioprospection
Enzymes
Protein sequencing
MALDI-TOF
Edman deg
cDNA Libraries
Sequencing of cDNA
Gene cloning
Glicosylome
Catalysis
Kinetic (Vmax, Km Kcat, etc)
Specificity (substrate/ product)
Studies of biophysics
Spectroscopy (CD, Fluorecense, DLS, FTIR, etc)
Expression Systems
E. coli
yeasts
Directed Evolution
X-Rays Cristalography

Research Group


Thank you