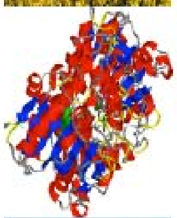
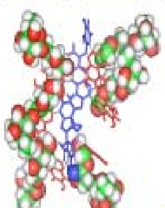
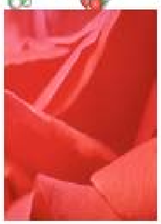



Biocatalysis for the production of chemicals and polymers: ***Synthesis of glucans and fructans.***

Agustin López Munguia
Instituto de Biotecnología, UNAM
Cuernavaca, Morelos.
México.

Instituto de Biotecnología

Campus Morelos - Universidad Nacional Autónoma de México

www.ibt.unam.mx

Academic staff and students

102 Researchers

- 12 Research Associates
- 36 Assistant Professors
- 25 Associate Professors
- 27 Full Professors
- 2 Emeritus

87 Technicians

43 Postdoctorals

326 Students

- 108 Undergraduate thesis
- 124 Masters
- 94 Ph.D.

42 research groups

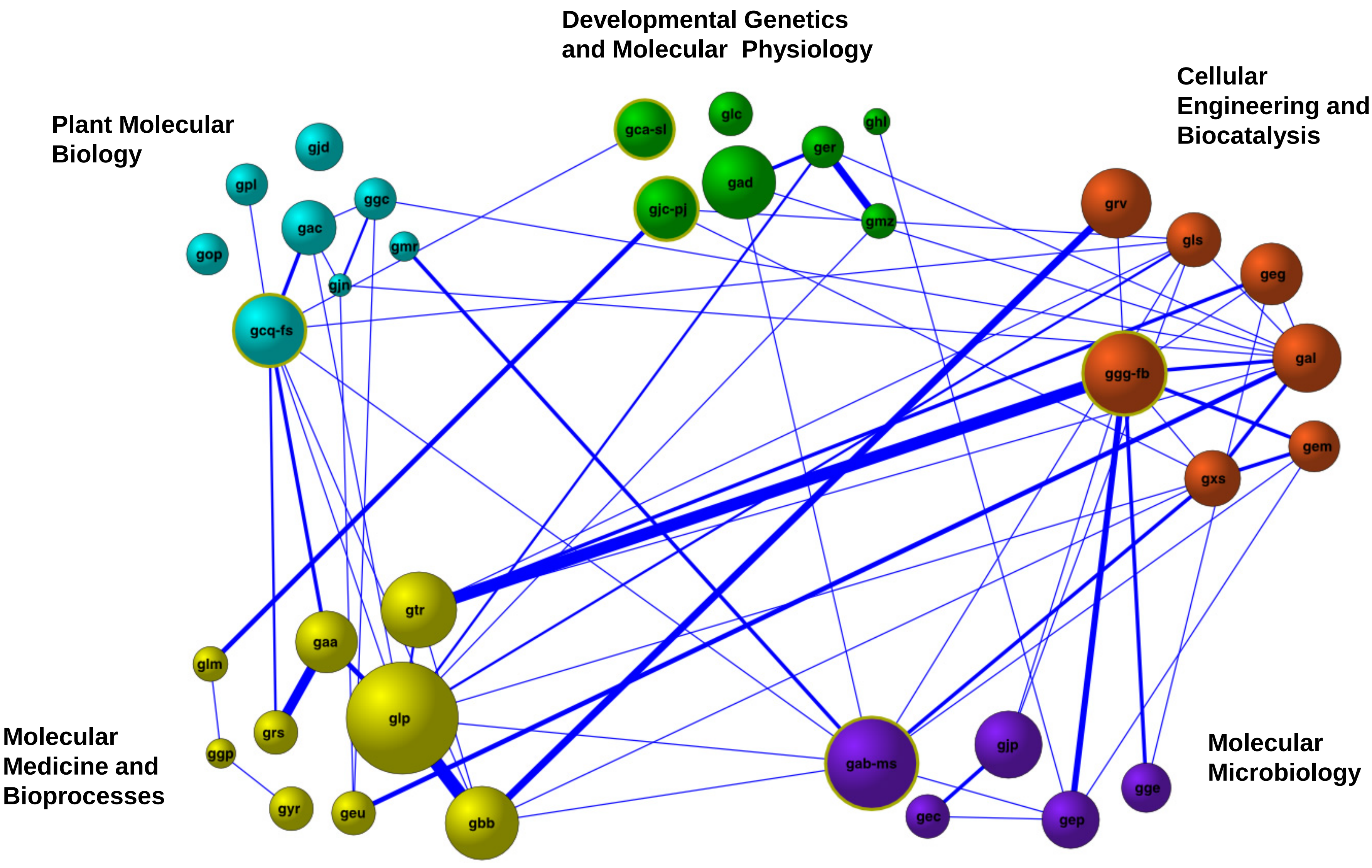
Departaments

- Plant Molecular Biology
- Developmental Genetics and Molecular Physiology
- Cellular Engineering and Biocatalysis
- Molecular Medicine and Bioprocesses
- Molecular Microbiology

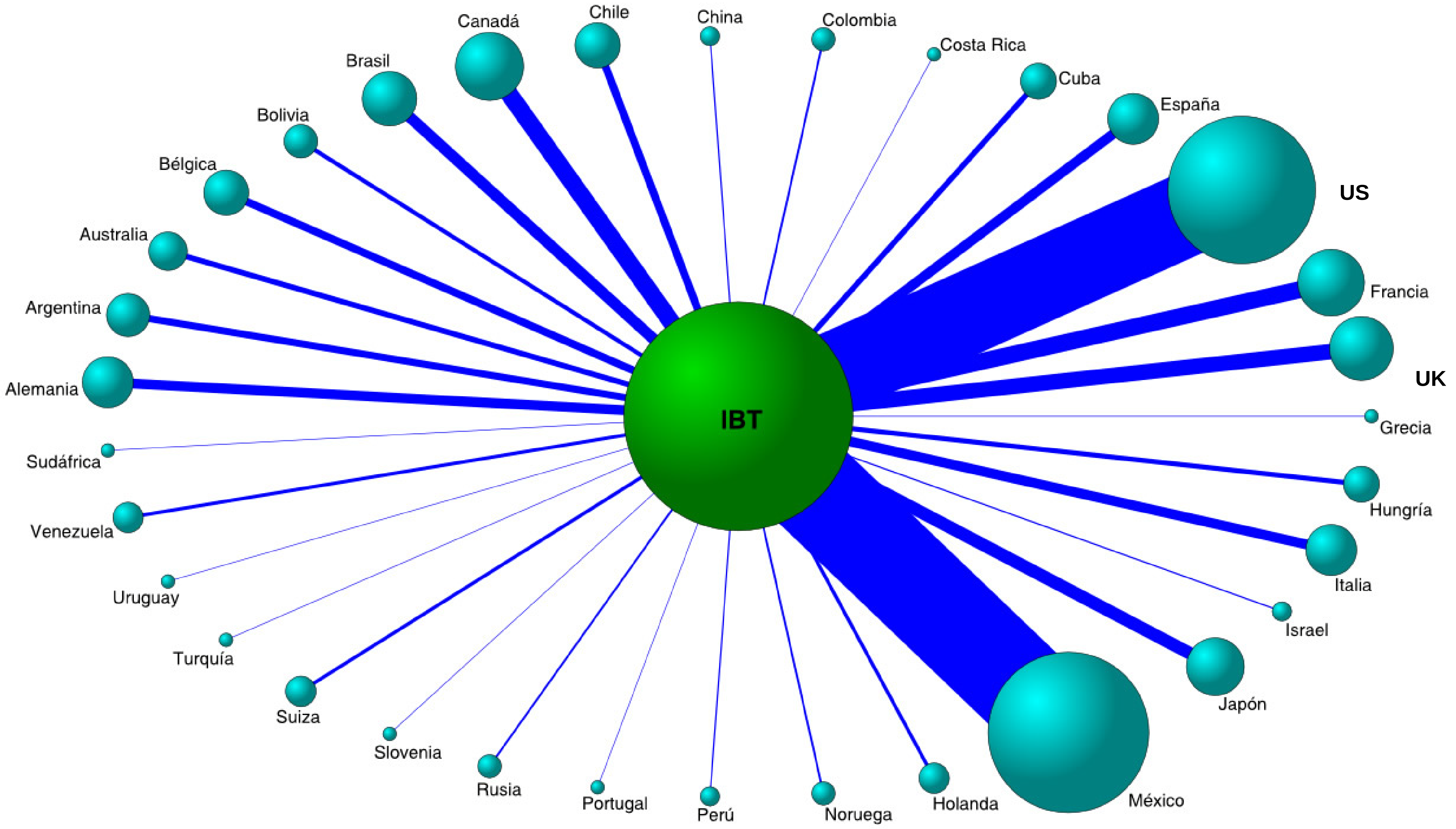
Technical core units

- Confocal and electron microscopy
- Pilot plant
- Synthesis and sequencing of DNA
- Plant tissue culture
- Animal house
- Transgenic rodents
- Proteomics (Orbitrap Velos, Orbitrap ETD)
- Massive sequencing of DNA (Illumina)
- Live cell microscopy and image processing (multiphotonic confocal microscopy, TIRFM)

Publications with coauthors from different departments



International Collaboration



Relationships with national and international industries

Silanes

Instituto Bioclon

Aventis Cropscience

Boehringer Ingelheim

CIDEC

Bayer CropScience

Protein Sciences CORPORATION

Production of microalgae

Birmex

DOMECQ

natura

FERRING PHARMACEUTICALS

bp

British Petroleum

DEBIOPHARM GROUP

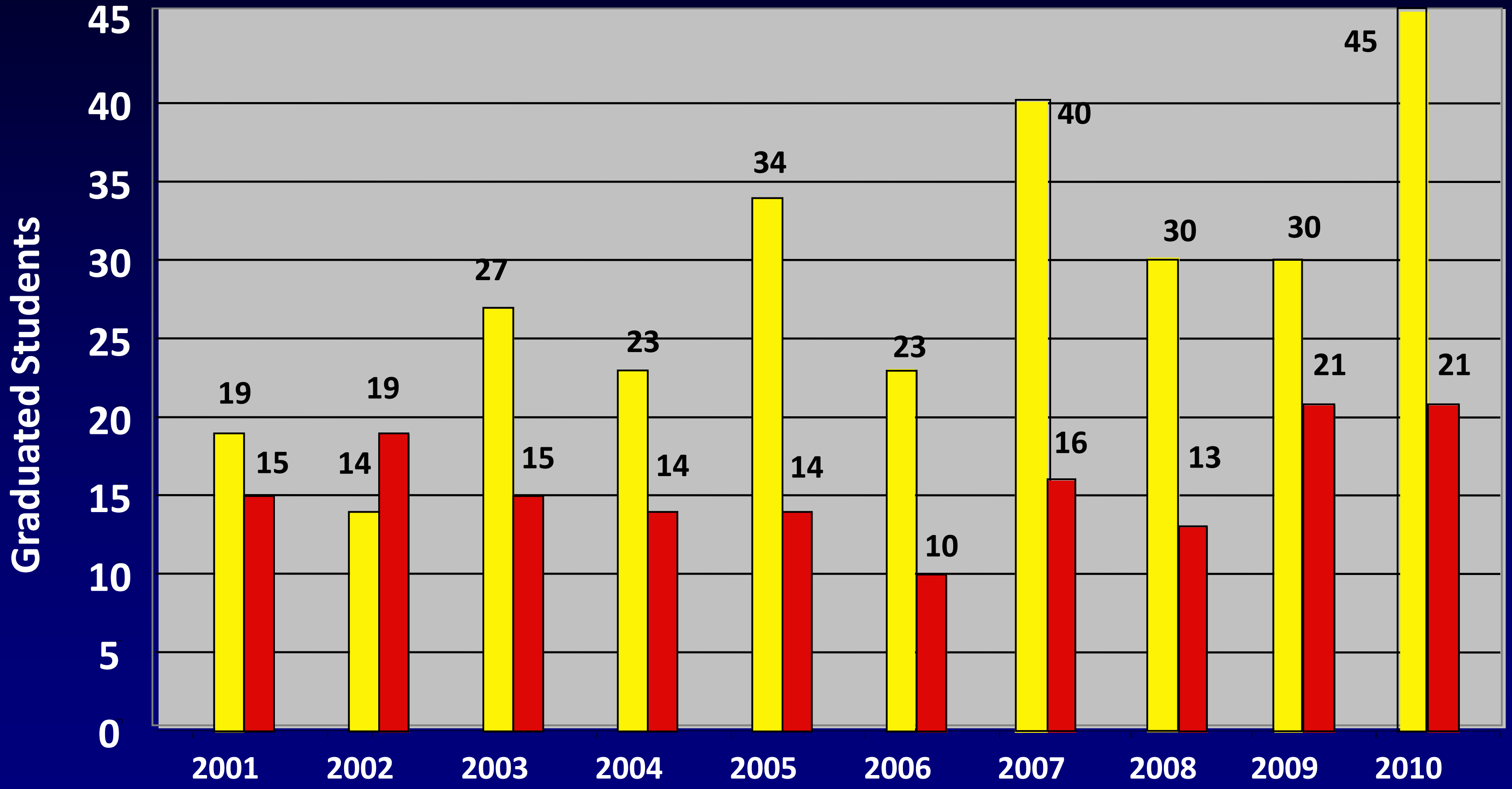
PIONEER. A DuPont Company

“spin off” companies
created since 2009

- **BIODETECTA**
  Molecular diagnostics
- **Agro & Biotecnia**
  Production of biofungicides
- **Peptherapeutics**
  Peptides with pharmacological action
- **Corporación Mexicana de Transferencia de Biotecnología**
  Formulation of Bt toxins for insect control
- **Biopolymex**
  Biological synthesis of plastics

Students graduated

Biochemical Sciences Program



Graduates/year
(2008-2010)

MSc = 35
PhD = 18

50 graduates/yr

Instituto de Biotecnología

2010

Molecular Medicine and Bioprocesses

Head of Dept: Tonatiuh Ramírez

Groups and research lines

Alejandro Alagón “Toxinology and antibody technolgy”

Lourival Possani “Scorpion toxins: structure and mechanisms of action”

Roberto Stock “Snake and spider venoms: development of therapeutic and profilactic measures”

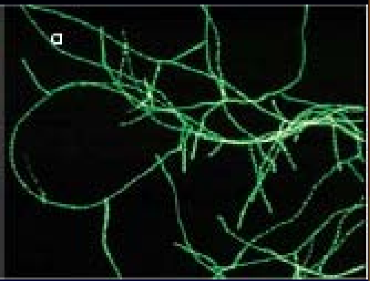
Baltazar Becerril “Development of phage-displayed antibodies for diagnostics and therapeutic uses”

Tonatiuh Ramírez “Engineering and integration of bioprocesses, production of recombinant proteins in eukaryotic and prokaryotic cell cultures”

Yvonne Rosenstein “Immune regulation of T-cell lymphocytes”

Leonor Pérez & Gustavo Pedraza “Molecular mechanisms controlling neuro inflammation”

Enrique Rudiño “Structural biochemistry of metalloproteins”

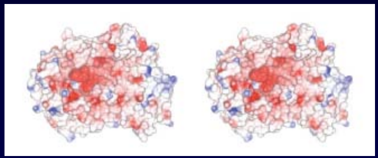


Instituto de Biotecnología

2010

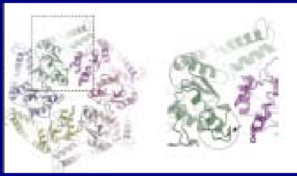
Cellular Engineering and Biocatalysis

Head of Dept: Enrique Galindo



Groups and research lines

Francisco Bolívar **Guillermo Gosset**	**"Cellular metabolism, microbial physiology and engineering of metabolic pathways in *E. coli*"**
Agustín López-M.	**"Engineering and biotechnology of enzymes"**
Enrique Morett	**"Evolution of the catalytic activity"**
Lorenzo Segovia	**"Structure-function relationship of enzymes"**
Xavier Soberón	**"Directed evolution of proteins"**
Rafael Vázquez	**"Environmental biotechnology and bioremediation"**
Enrique Galindo	**"Characterization and development of microbial bioprocesses: hydrodynamics, scaling up and recovery "**



The calalytic power of enzymes

- **A broad range of catalytic activities**
  on natural and non natural substrates
  in aqueous and non aqueous media

- **Virtually any enzyme can be produced and ameliorated.**

- **Mild reaction conditions**
  temperature, pressure, pH

- **Reaction selectivity**
  chemo-, regio, enantioselectivity

- **Less waste (no biomass waste)**
  E-factor vs fermentation , improved LCA

* **No activation, protection or deprotection reactions**

Innovative biomolecules

Novel molecular architectures not/hardly synthesized by chemistry

Innovative bioprocesses

Fewer steps (no protection/deprotection)

Less toxic/hazardous reagents & solvents

…. by sustainable conversion processes

- Reduction of toxic reagents, solvents and metal catalysts
- Reduction of energy and water consumption
- Reduction of waste and CO_2 production

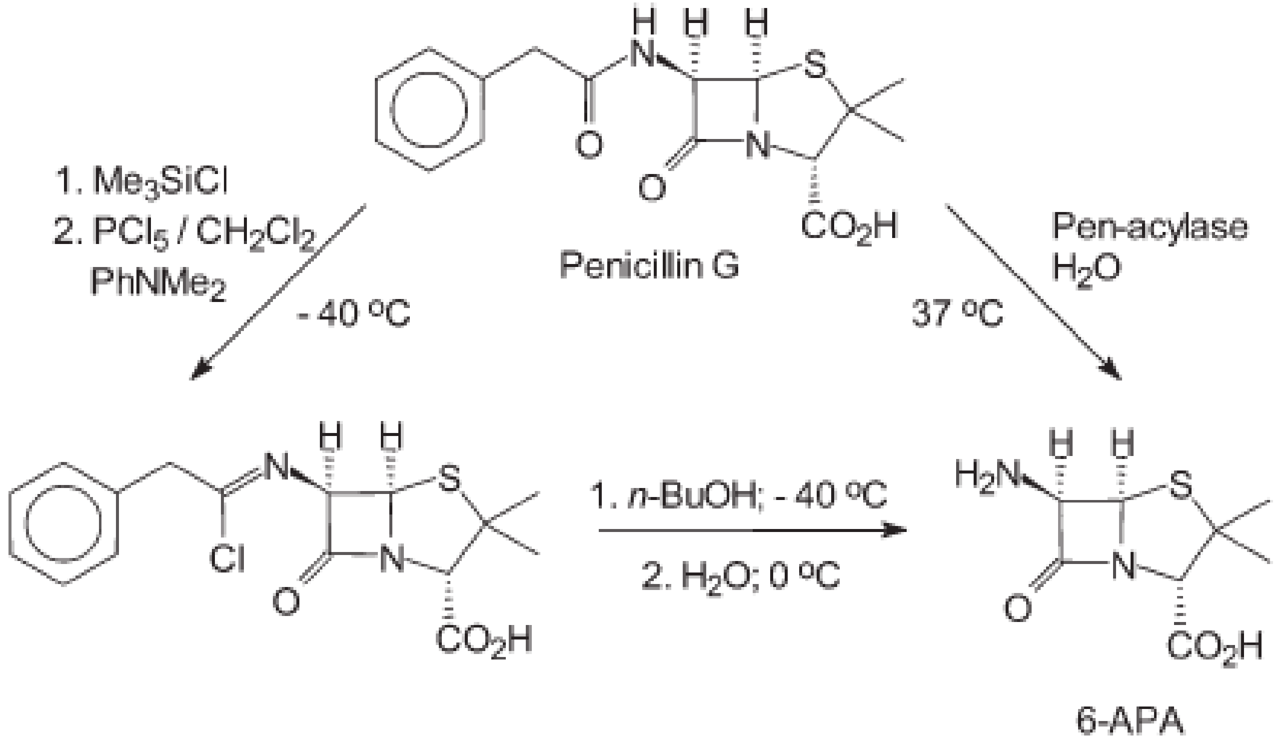
White Biotechnology / Green chemistry

Reduce process E-factor

$$\text{E-factor} = \frac{\text{by-products}}{\text{desired product}}$$

Table 1 E factors in the chemical industry

Industry segment	Product tonnage	E Factor (kg waste/kg product)
Oil refining	10^6–10^8	<0.1
Bulk chemicals	10^4–10^6	<1–5
Fine chemicals	10^2–10^4	5–50
Pharmaceuticals	10–10^3	25–100



1980's

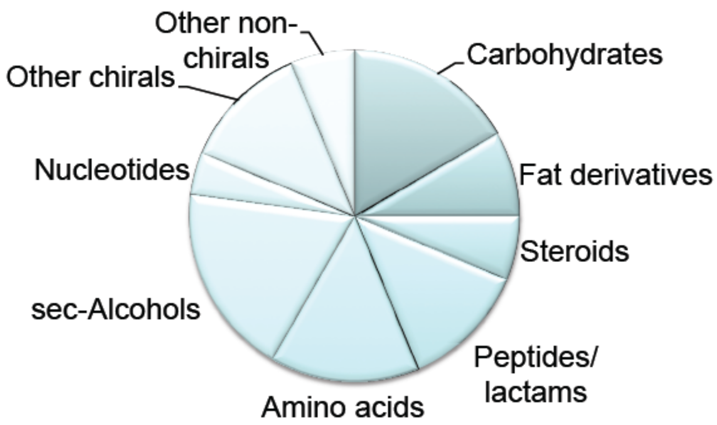
0.6 Kg Me_3SiCl
1.2 Kg PCl_5
1.6Kg $PhNMe_2$
0.2Kg NH_3
8.4 L nBu-OH
8.4 L CH_2Cl_2

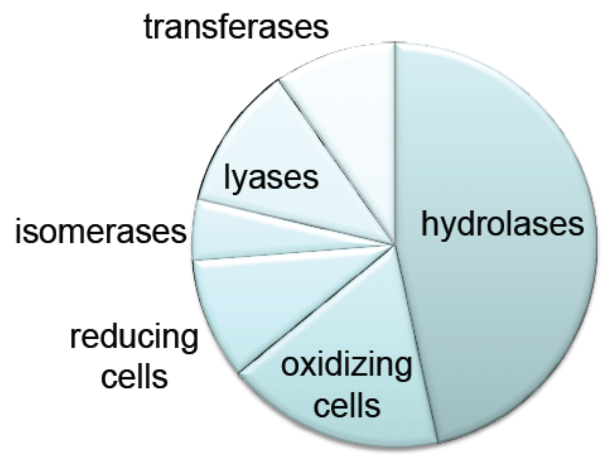
1Kg 6-APA

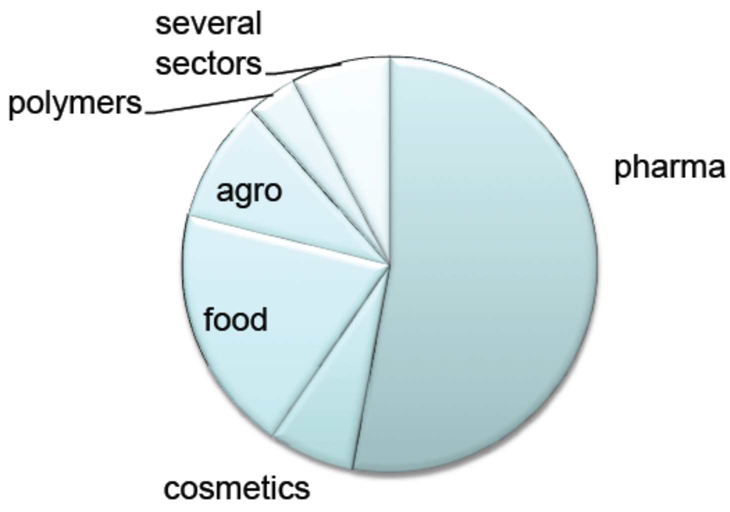
Higher Atom Economy, among other advantages
Chlorinated hydrocarbon solvents (CH_2Cl_2)

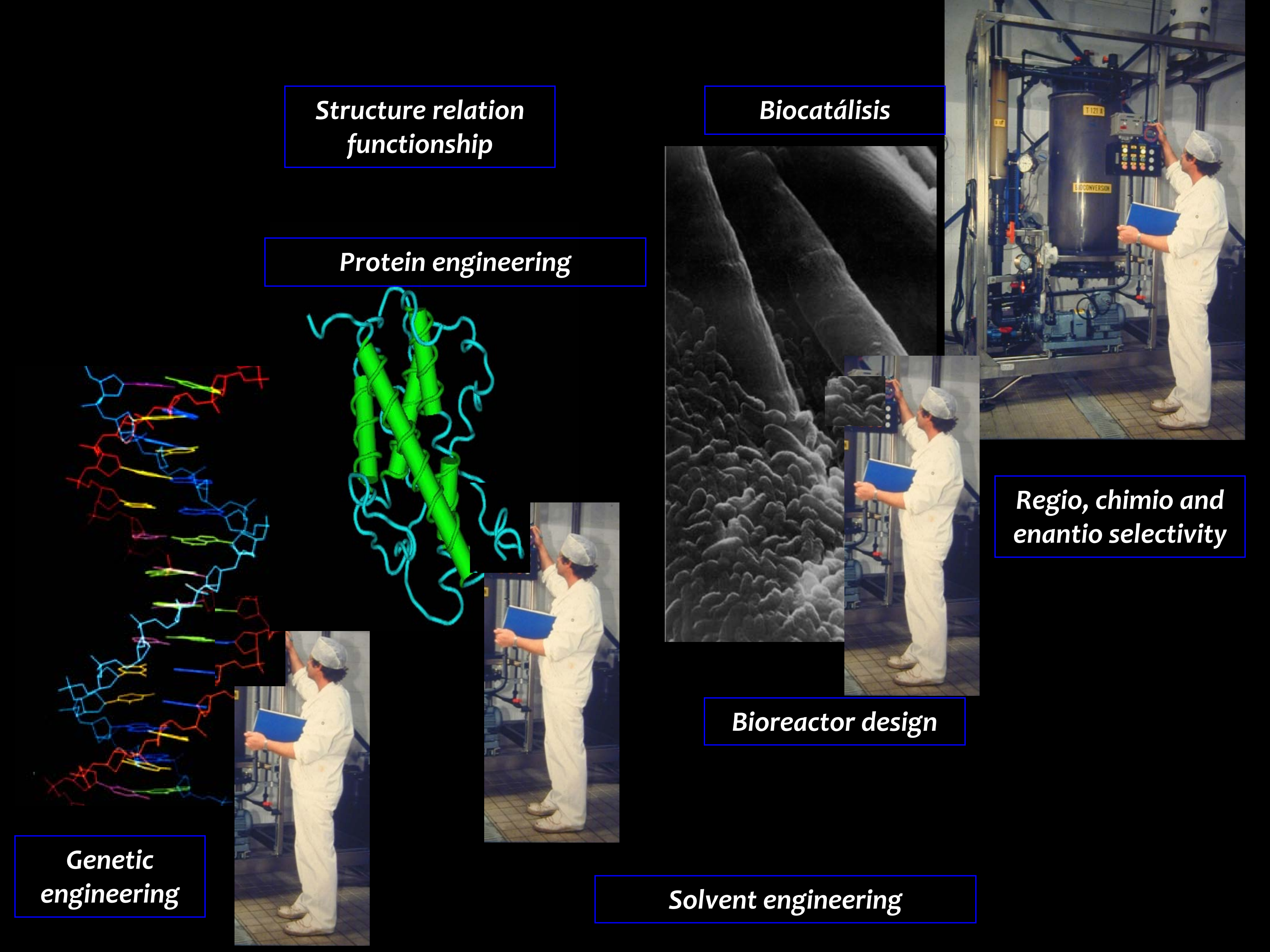
Industrial biocatalysts

More than 150 industrial biocatalytic processes on a scale from 1 to more than 10,000 tons/year








Structure relation functionship
Biocatálisis
Protein engineering
Regio, chimio and enantio selectivity
Bioreactor design
Genetic engineering
Solvent engineering

Marigold Carotenoids Extraction

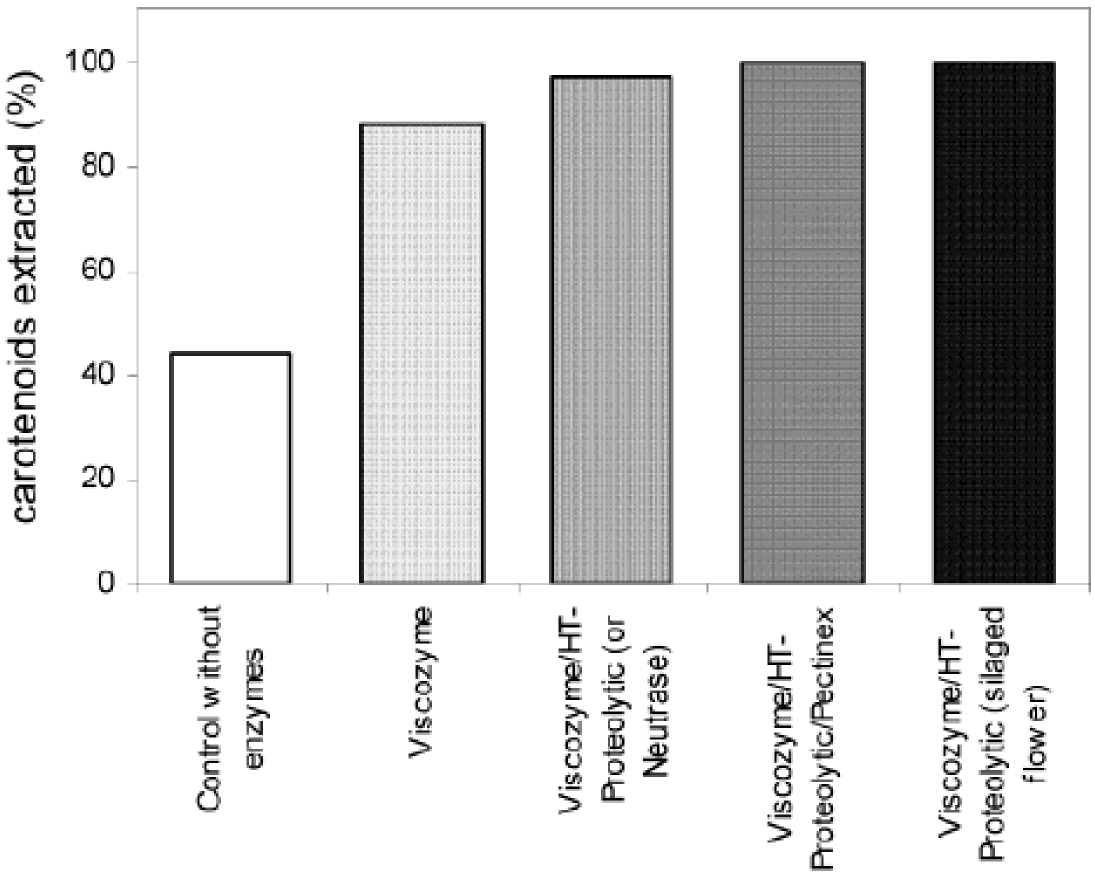
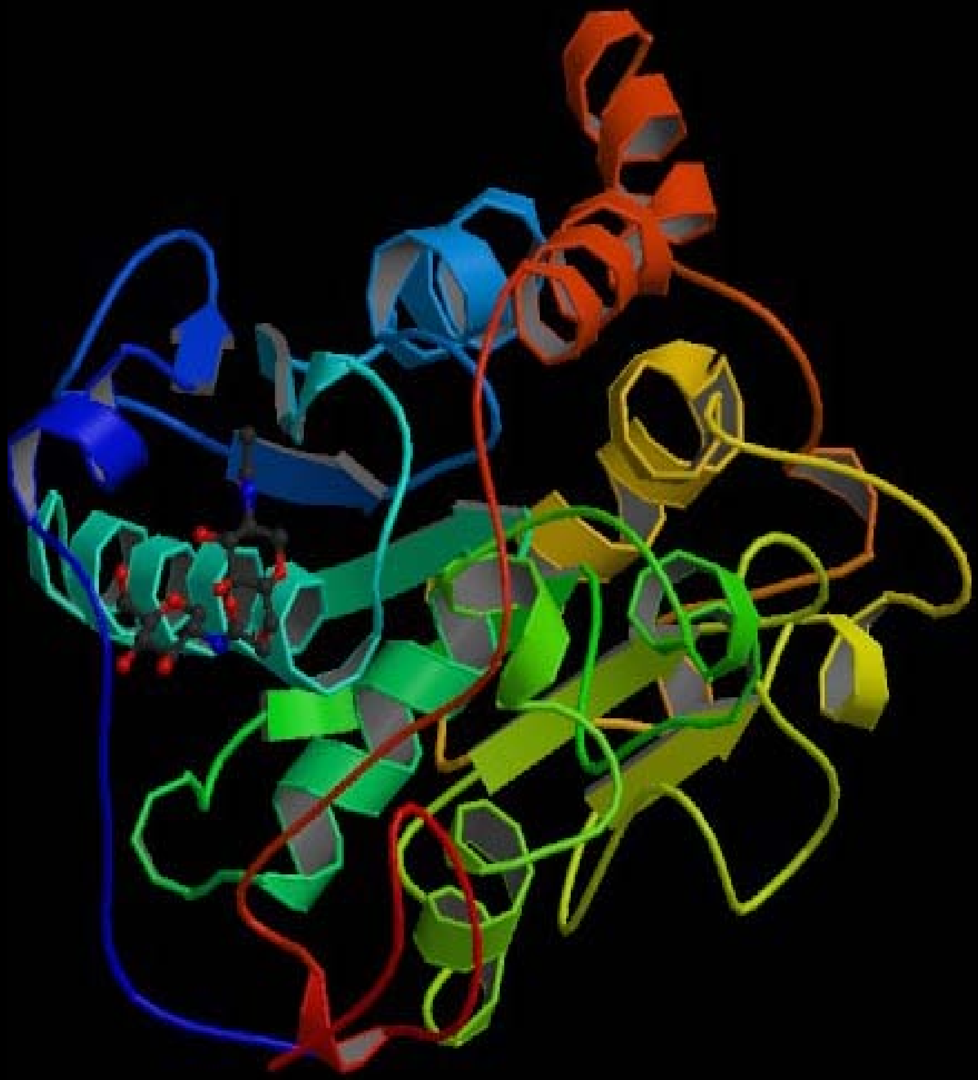
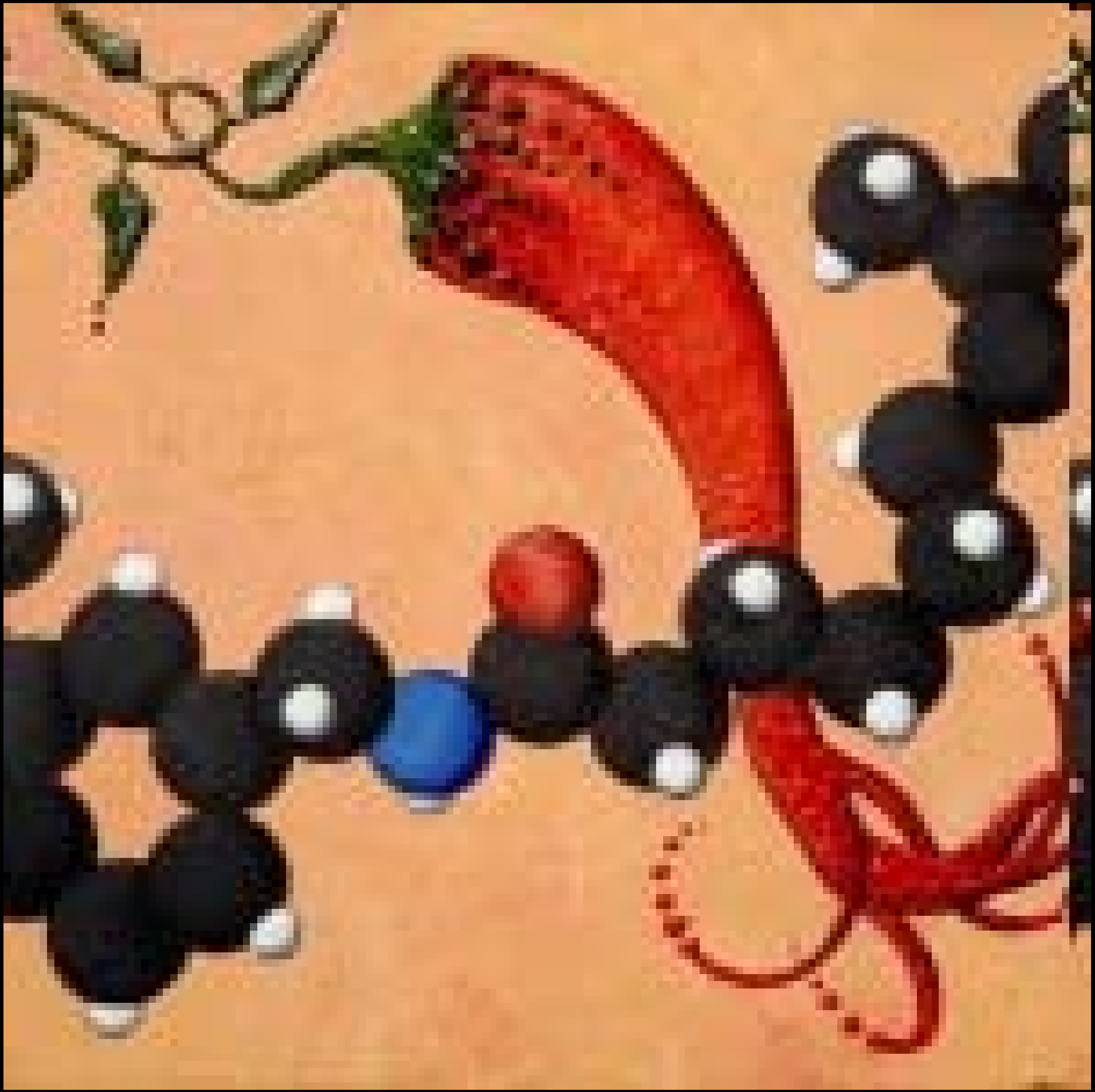
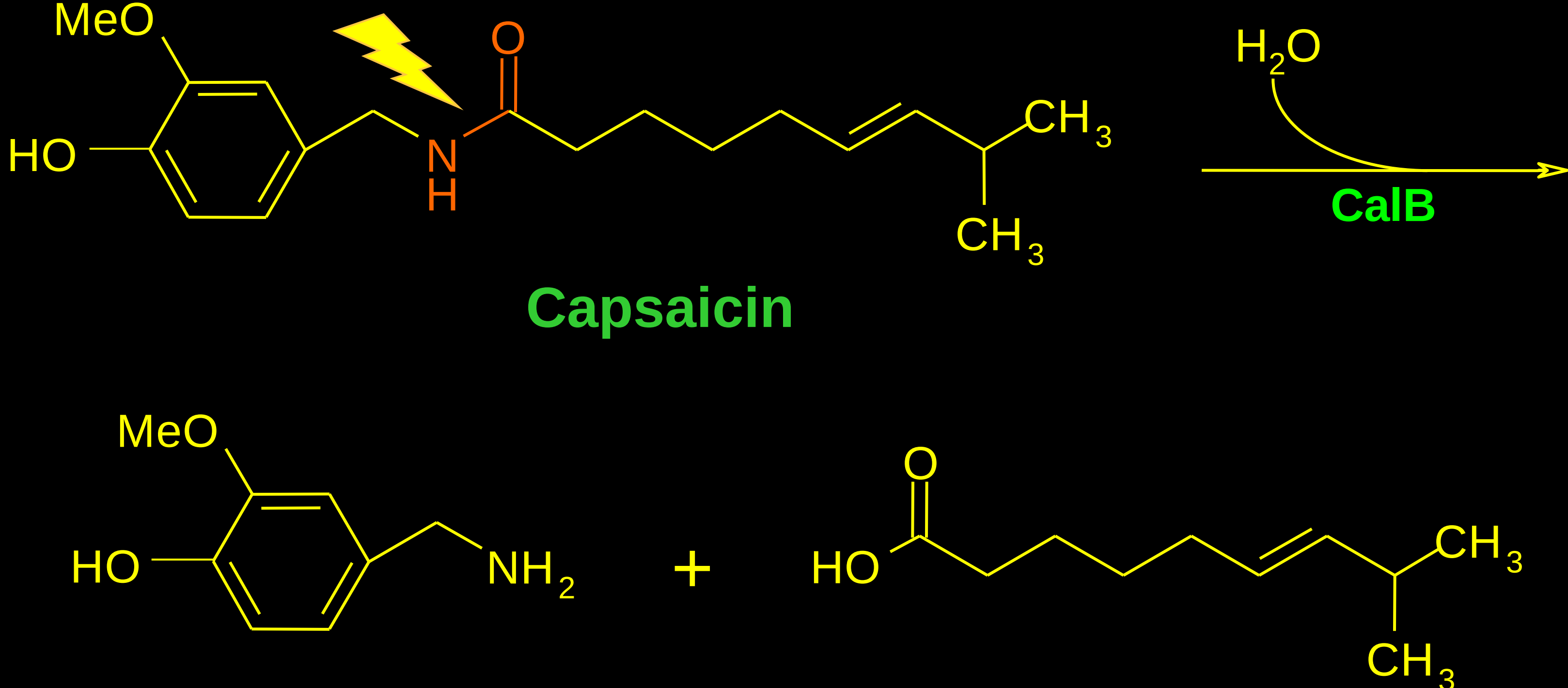


Figure 1. Effect of different commercial enzymes on the yield of carotenoids extracted from fresh marigold flowers. All enzymes were added at 1% (v or w/w flower depending on the enzyme presentation). All extractions were carried out with 32 mL of hexane and a 1:4 flower: solvent ratio for 24 h at 25 °C.

CalB

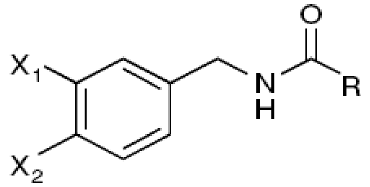






Vainillinamine **8-methyl-6-*trans*-nonanenoic acid**

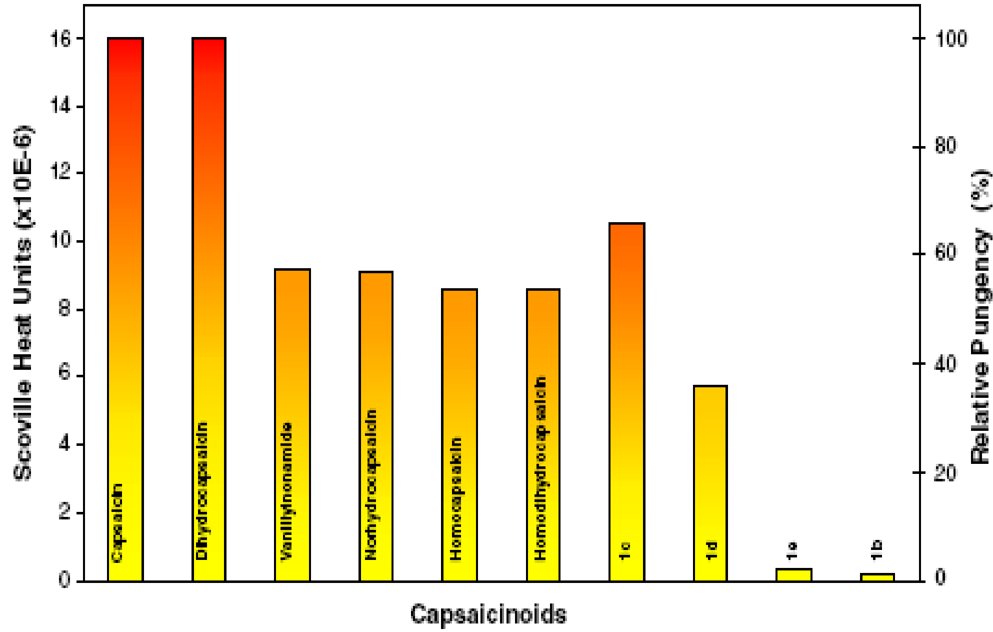
Reyes- Duarte D., *Castillo E. Bárzana E. & López-Munguía A. Biotechnology Letters. 22 :1811-1814, 2000.*



Compound	X_1	X_2
1	MeO -	HO -
2	H -	H -
3	MeO -	H -

Compound	R
a	$-C_3H_7$
b	$-C_5H_{11}$
c	$-C_7H_{15}$
d	$-C_9H_{19}$
e	$-C_{11}H_{23}$
f	$-C_{13}H_{27}$
g	$-C_{15}H_{31}$
h	$-(CH_2)_7CH{=}CH(CH_2)_7CH_3$

Scheme 2. Lipase-catalyzed synthesis of capsaicin analogues using different amines and acyl donors as substrates (see M&M section for synthesis conditions).



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ELSEVIER
Food Chemistry 100 (2007) 1202–1208
Food Chemistry
www.elsevier.com/locate/foodchem

Lipase-catalyzed synthesis of pungent capsaicin analogues

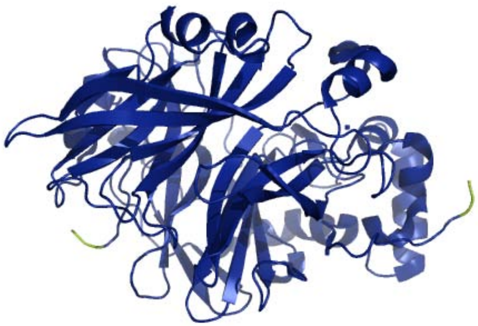
Edmundo Castillo [a], Alejandro Torres-Gavilán [a], Patricia Severiano [b], Navarro Arturo [b], Agustín López-Munguía [a,*]

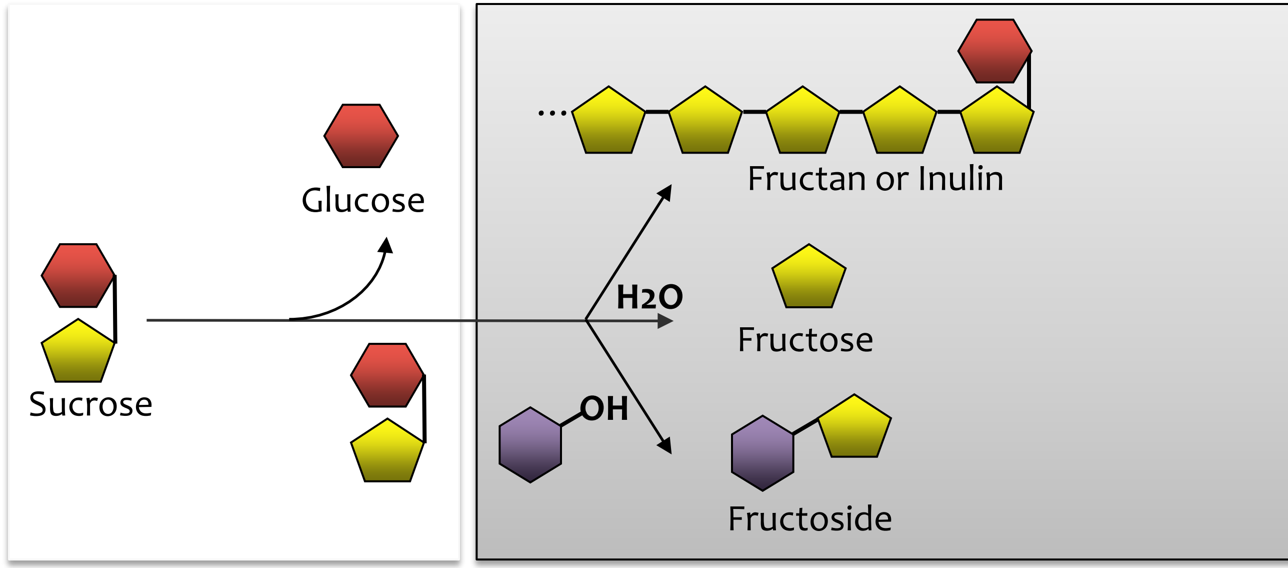
[a] *Departamento de Ingeniería Celular y Biocatálisis, Instituto de Biotecnología, Universidad Nacional Autónoma de México, Apartado Postal 510-3, Cuernavaca, Morelos, CP 62271, Mexico*

[b] *Facultad de Química, Universidad Nacional Autónoma de México, Cd. Universitaria, CP 04510 México, D.F., Mexico*

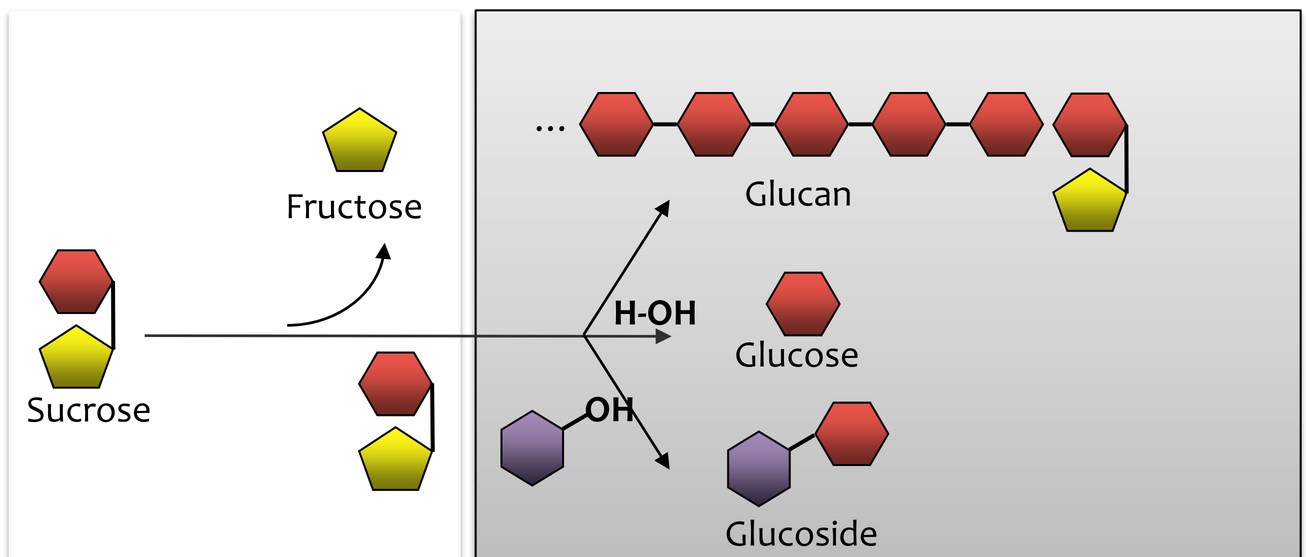
Received 13 September 2005; received in revised form 8 November 2005; accepted 29 November 2005

FRUCTANSUCRASES or FTFs



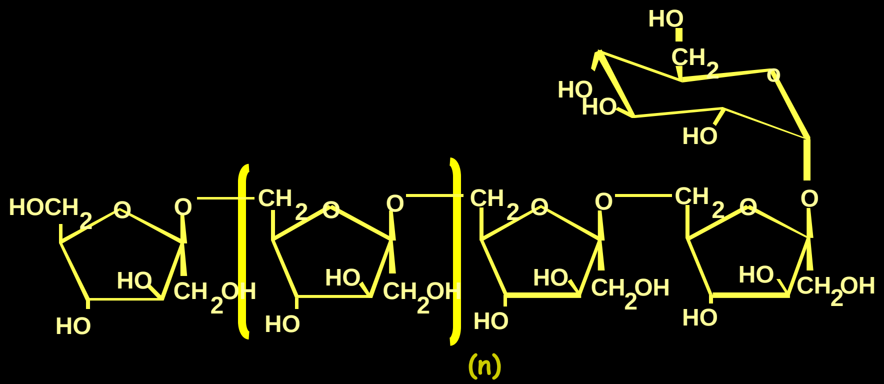


GLUCANSUCRASESor GTFs

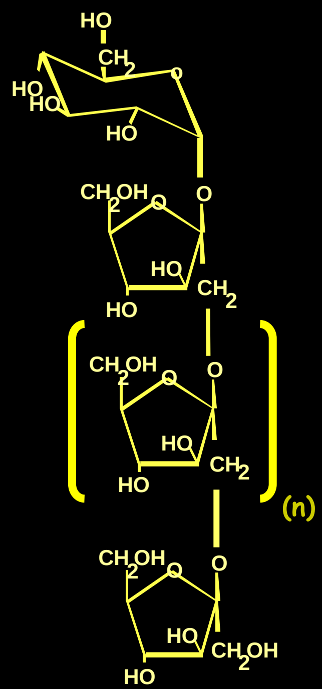


Levan

Many monocotyledons &
Bacillus spp., E. amylovora, Z. mobilis…



ß-2,6 linked fructosyl moieties with branching through ß-2,1 linkages



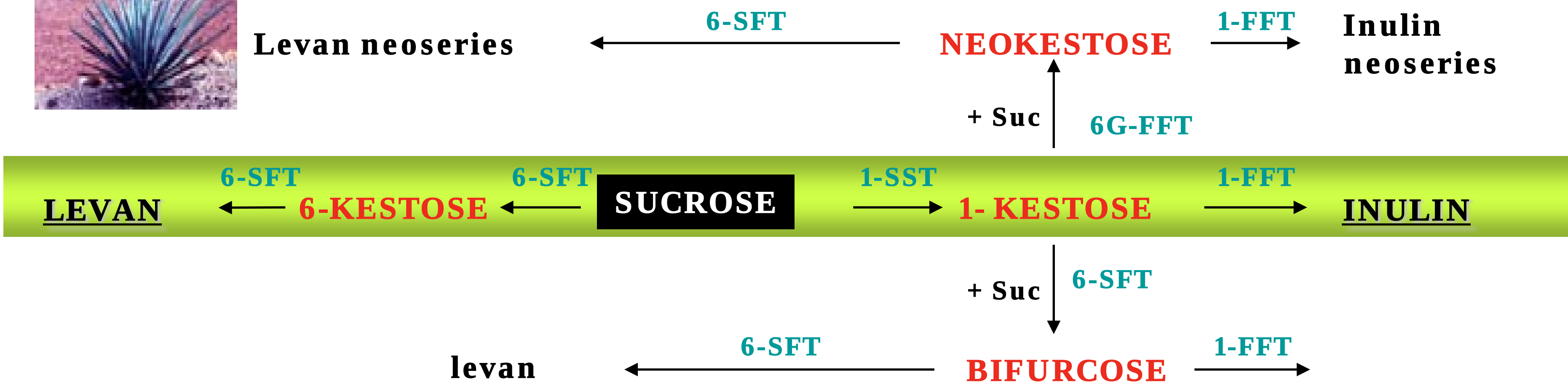
Inulin

Mainly in dicotyledons, fungal and *S. mutans*

ß-2,1 linked fructosyl moieties with branching through ß-2,6 linkages

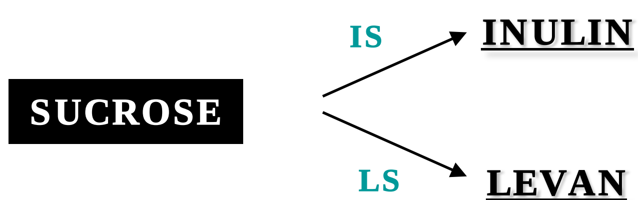
Fructansucrases

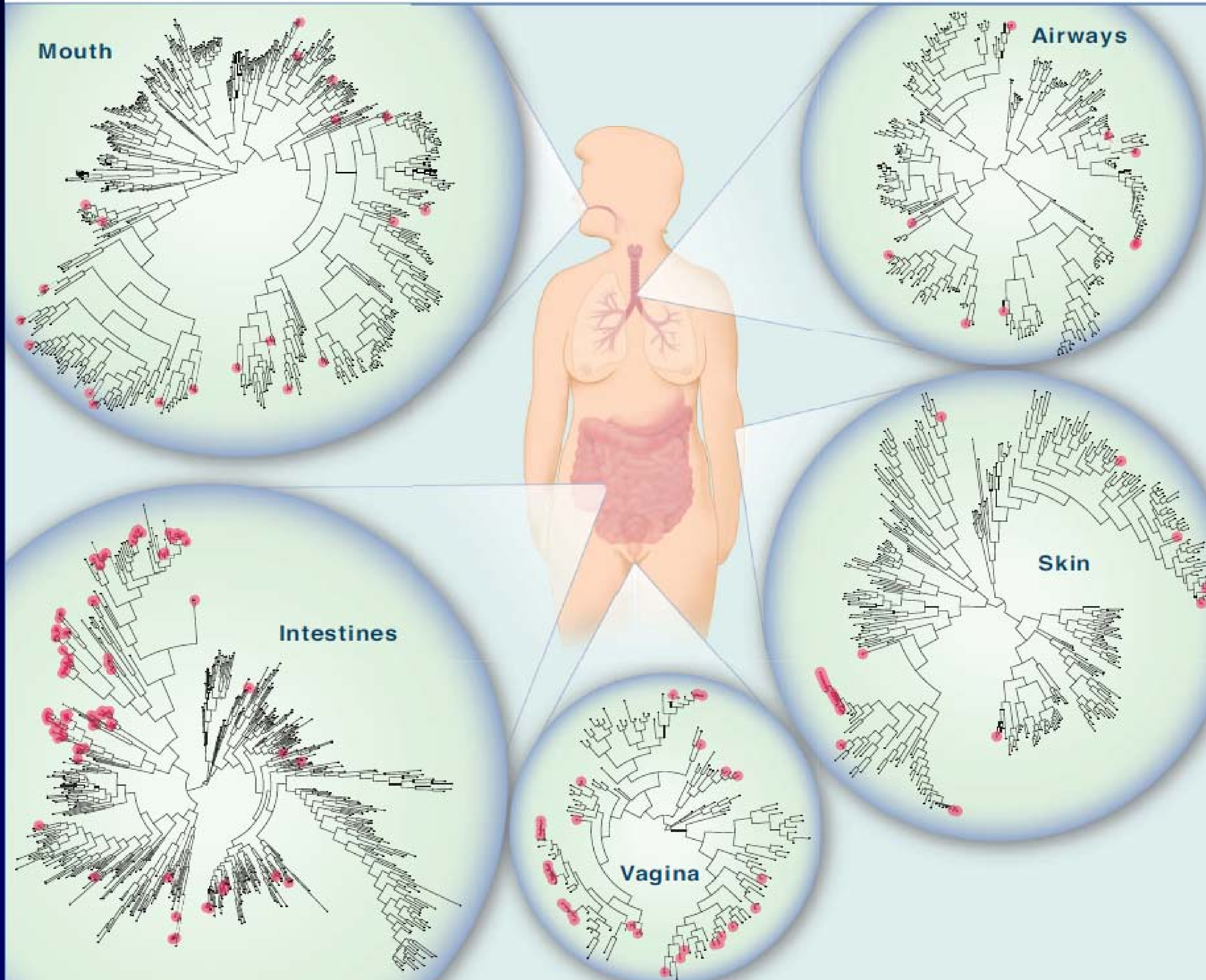
- **Plants**



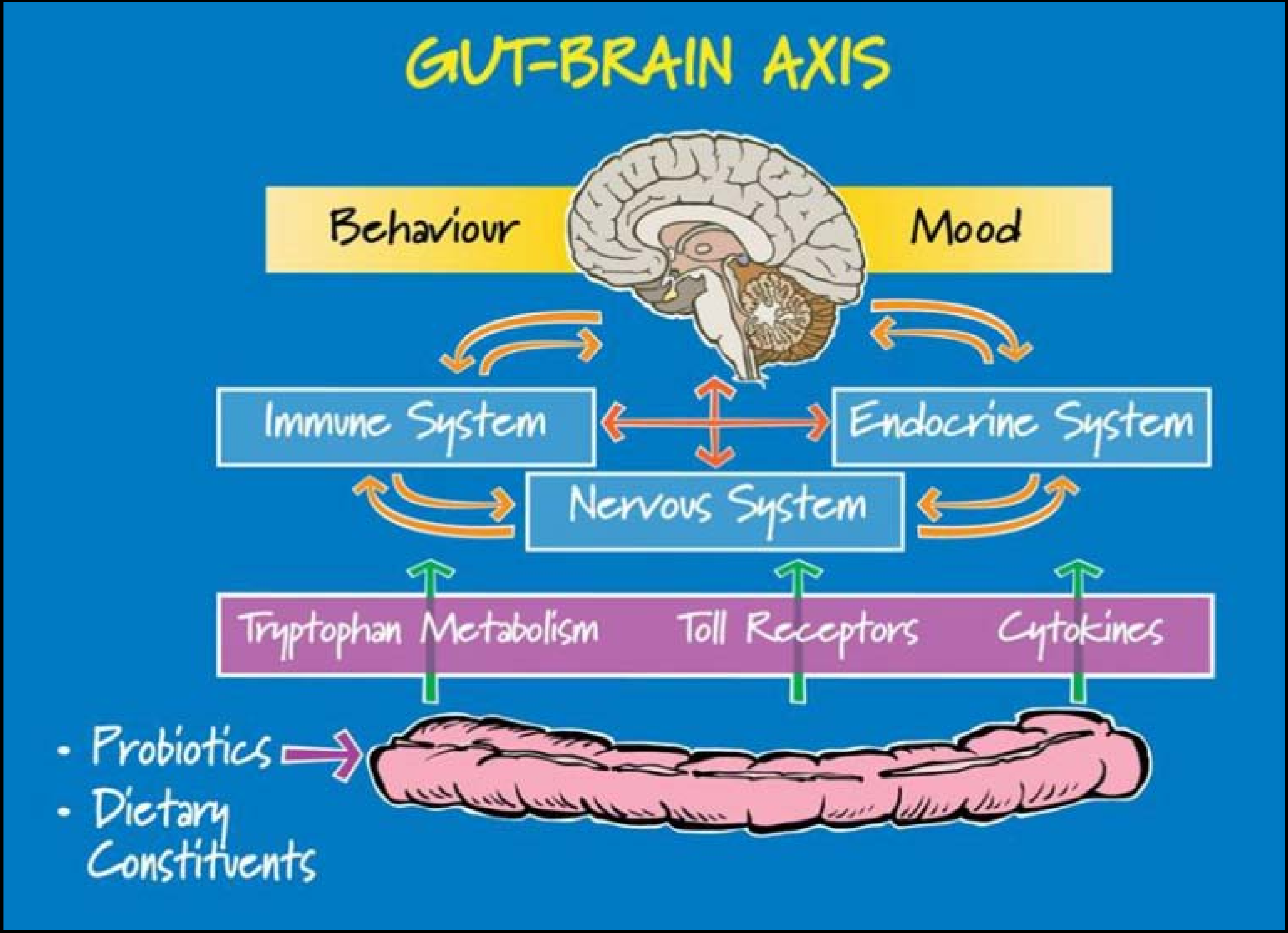
(Vijn y Smeekens, 1999).

- **Bacteria**





… humans are superorganisms whose methabolism represents an amalgamation of microbial and human attributes…..


GUT-BRAIN AXIS
Behaviour
Mood
Immune System
Endocrine System
Nervous System
Tryptophan Metabolism
Toll Receptors
Cytokines
• Probiotics
• Dietary Constituents

Sociomicrobiology

an emerging field…..

elucidate how we humans can remain on friendly terms with our microbiota

E. Peter Greenberg
University of Washington

…..You would basically not expect a facet of human biology not to be influenced in some either direct or indirect way by what the microbiota is doing….. A susrainable relation with our microbiota!!!!

Table 5. Summary and conclusion on the prebiotic effect of various oligosaccharides

Carbohydrate	Non-digestibility	Fermentation	Selectivity	Prebiotic status
Inulin	Yes	Yes	Yes	Yes
Transgalacto-oligosaccharides	Probable	?	Yes	Yes
Lactulose	Probable	?	Yes	Yes
Isomalto-oligosaccharides	Partly	Yes	Promising	No
Lactosucrose	NA	NA	Promising	No
Xylo-oligosaccharides	NA	NA	Promising	No
Soyabean oligosaccharides	NA	NA	NA	No
Gluco-oligosaccharides	NA	NA	NA	No

?, Preliminary data, but further research still needed; NA, data not available.

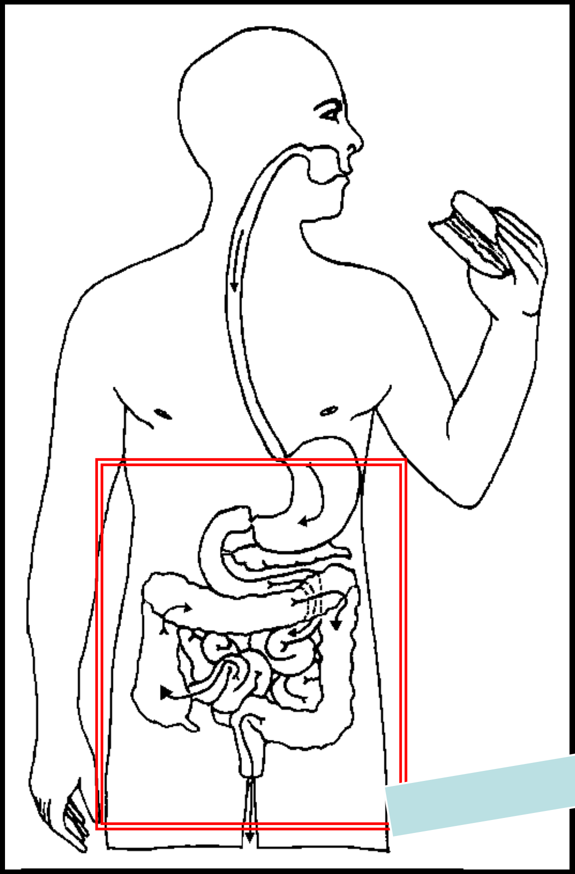
Three criteria define a Prebiotic

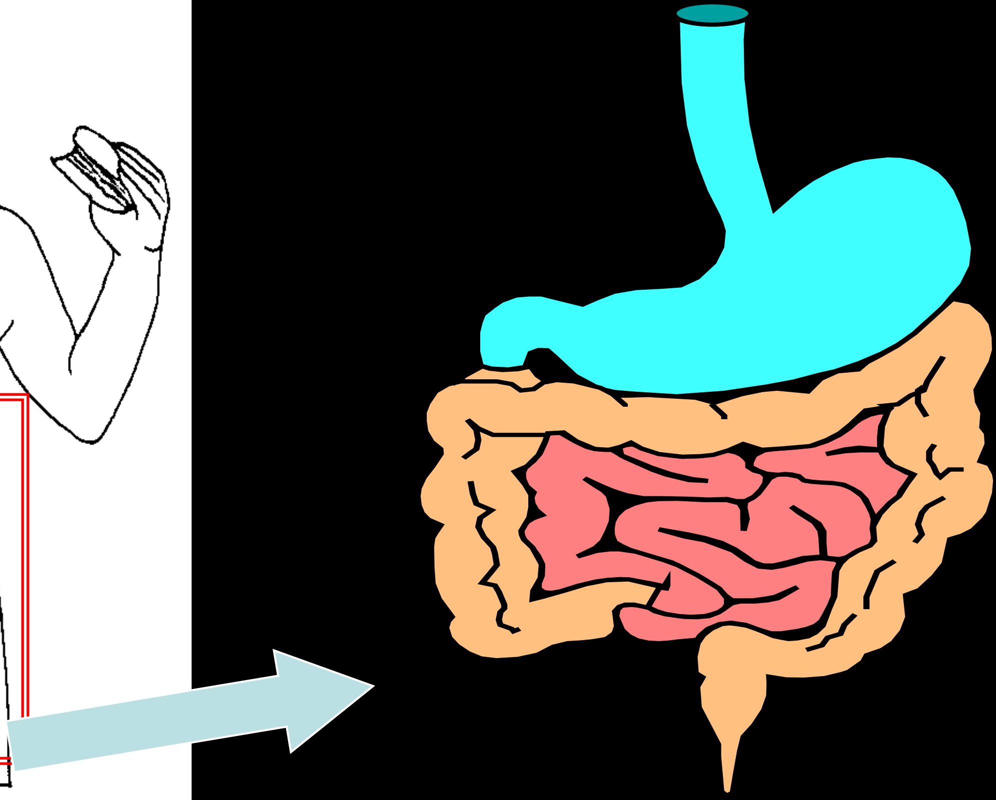
Gibson et al: **Dietary modulation of the human colonic microbiota: updating the concept of prebiotics** *Nutrition Research Reviews* (2004), 17, 259–275

(a) resistance to gastric acidity, hydrolysis by mammalian enzymes and gastrointestinal absorption;

(b) fermentation by intestinal microbiota;

(c) selective stimulation of the growth and/or activity of intestinal bacteria associated with health and well being.

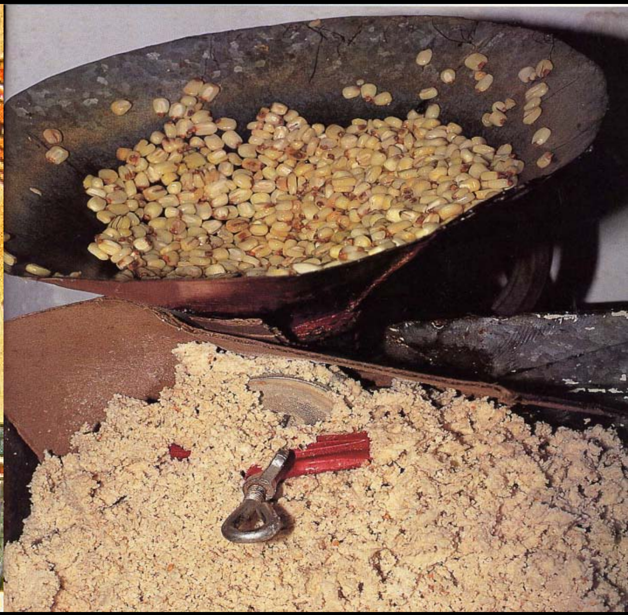




XX: bacteria as food
XXI: food for bacteria

Little effort has been paid to study the role of microbial fructans associated to the mexican diet.





Pulque

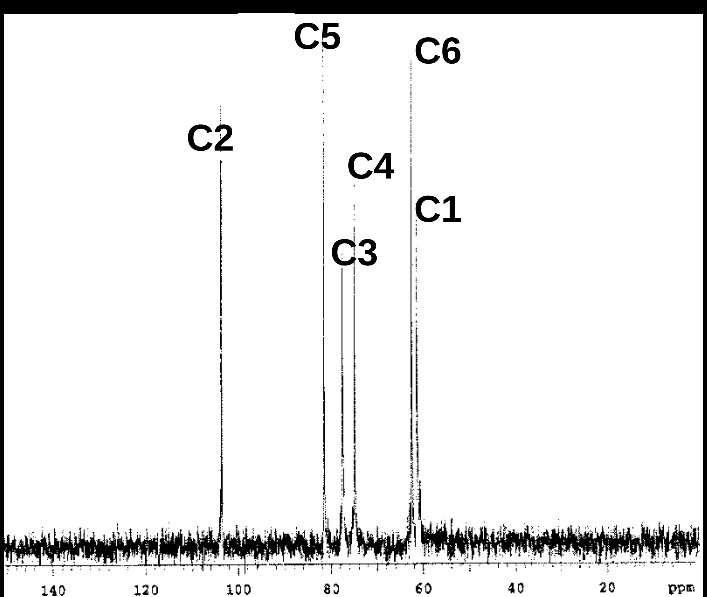
Pozol

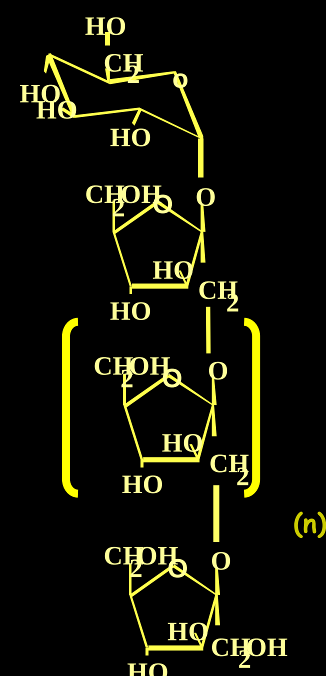
Bioprospection

Pozol

Screening for *Leuconostoc mesenteroides* cells and enzymes with glucosyltransferase activity (CW collection)

Strain CW28 identified as *Leuconostoc citreum* by 16S RNA with a cell associated fructansucrase activity 170 kDa



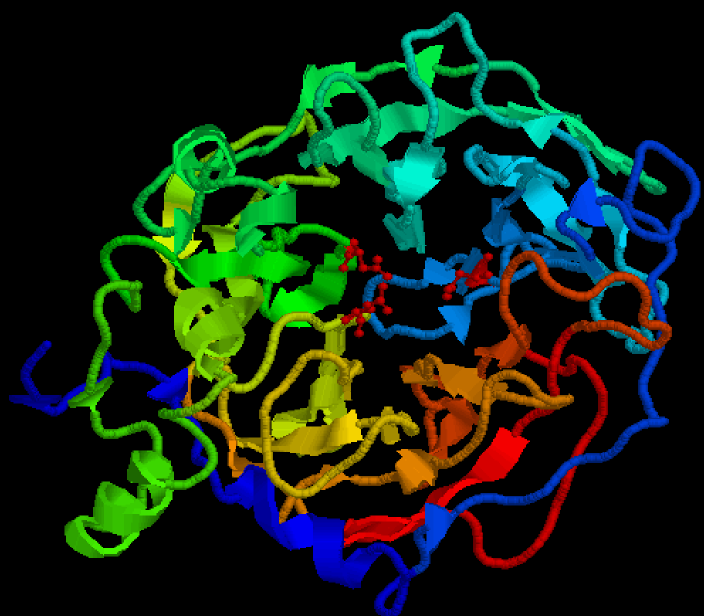


Leuconostoc citreum CW28 Inulosucrase (IslA)

N-terminal		Catalytic Domain	C-terminal	
ASR		FTF *Sac B*	ASR	
40%	?	45%	31%	80%

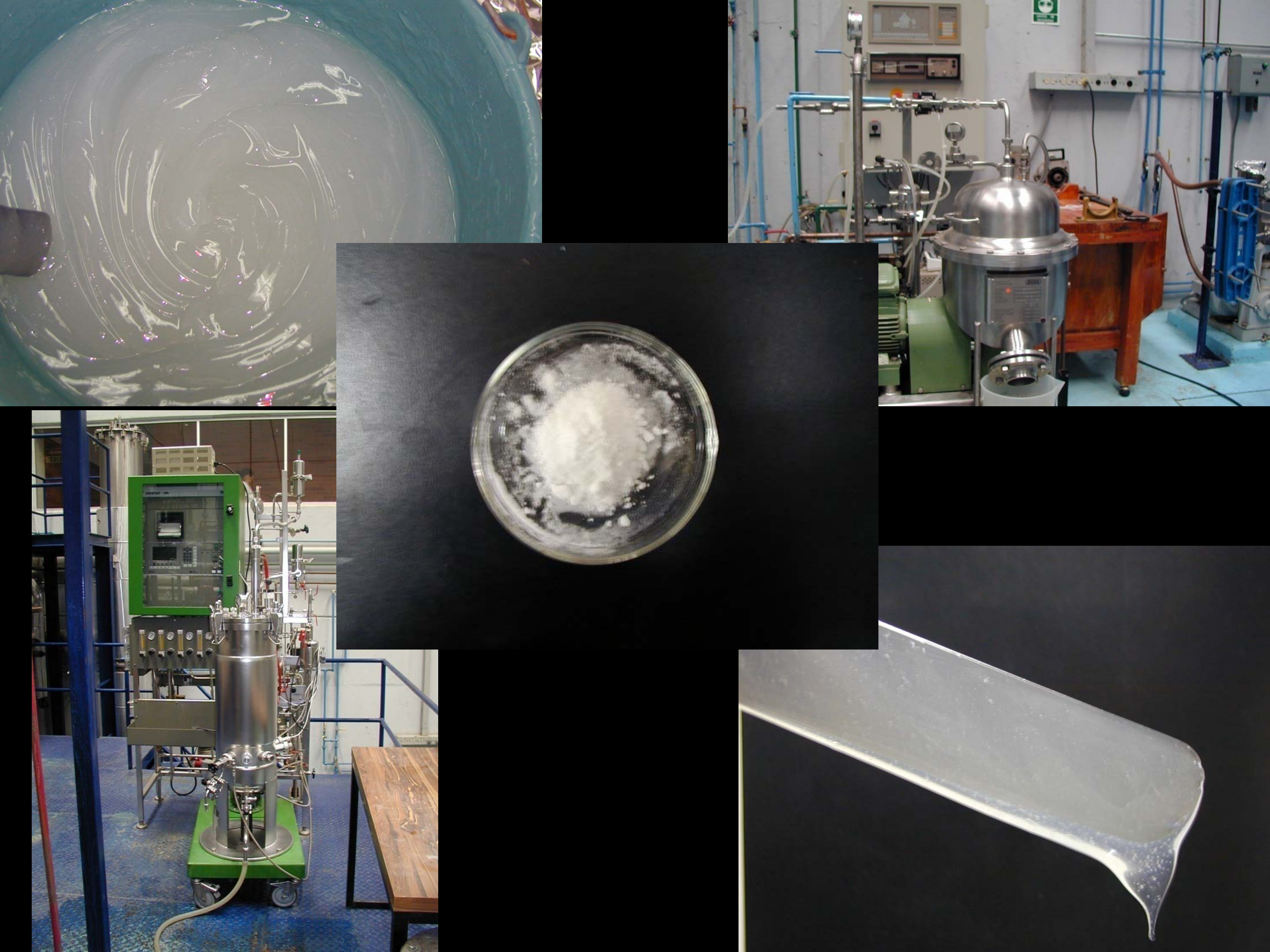
1 209 734 940 1490

5 bladed β-propeller

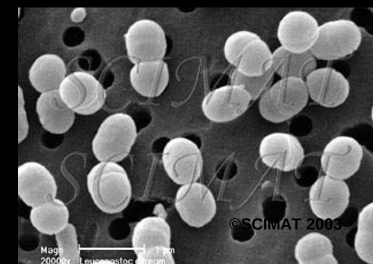


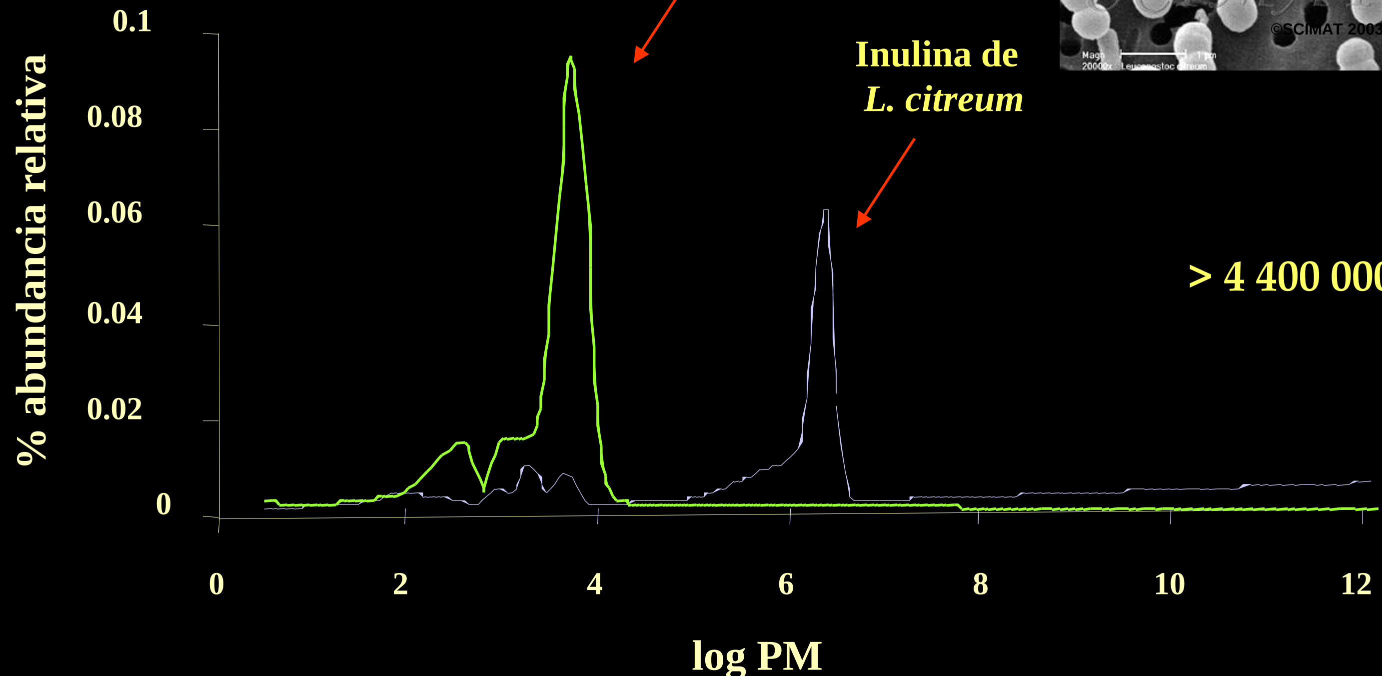
ASR (alternansucrase), a glucansucrase from L mesenteorides NRRL B-1355

Olivares-Iliana V, López-Munguía A & Olvera C. *J Bacteriol* 2003. 185: 3606-3612.



176 – 22 308 Da

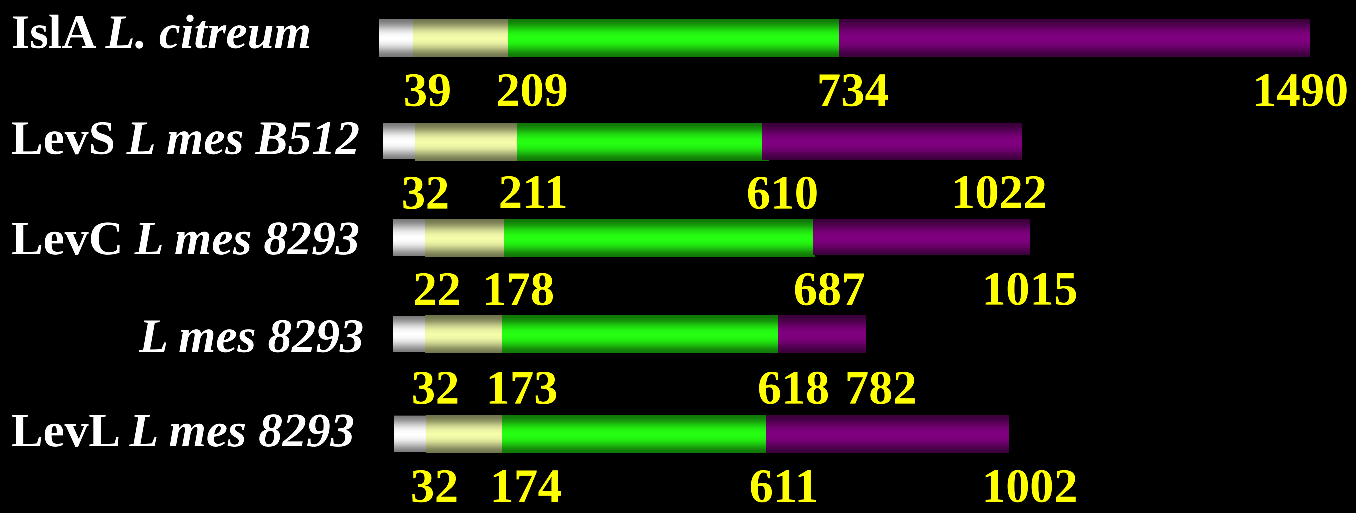




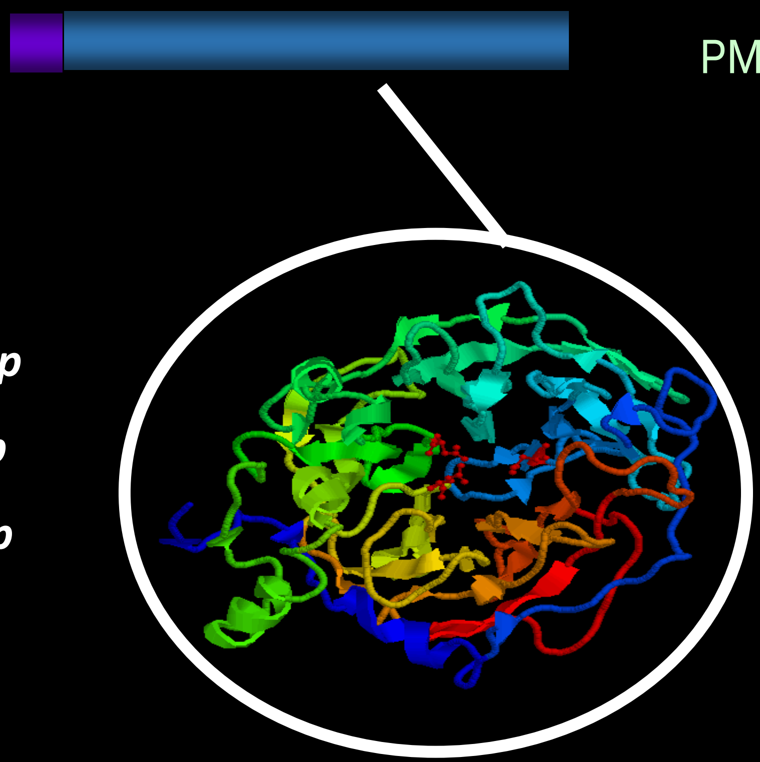
> 4 400 000 Da

Protein	Function	Accesion number	Gene size	Total aa number	Molecular weight
Lmes02002054	Levansucrase	ZP_00064342.1	3048bp	1015aa	113 kDa
Lmes02002055	Levansucrase	ZP_00064343.2	2345 bp	783aa	87 kDa
Lmes02002058	Levansucrase	ZP_00064346.1	3009 bp	1002aa	112 kDa

A new subfamily of Fructansucrases



Bacillus subtilis LEVANSUCRASE ***(SacB)***



PM : 40 a 100 kDa

Gram positive bacteria

Streptococcus spp

Leuconostoc spp

Lactobacillus spp

Gram negative bacteria

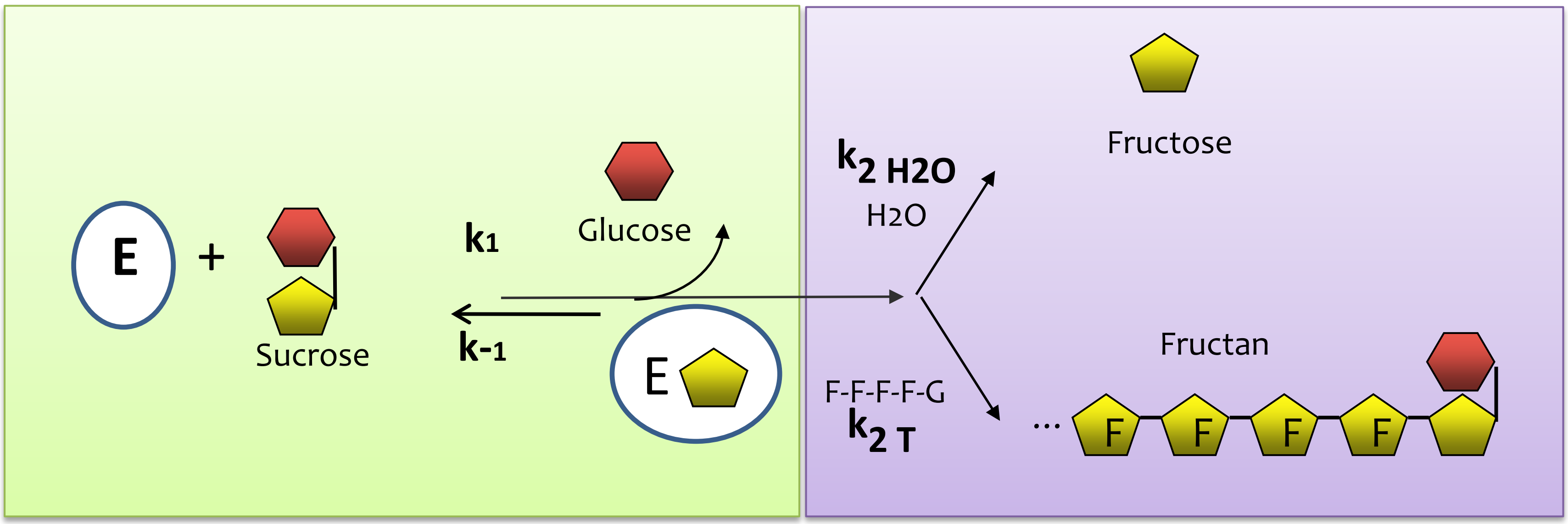
Gluconacetobacter diazotrophicus

Zymomonas mobilis

Rahnella aquatilis

- Polymer type (ß 2-1 o ß 2-6),
- Branching,
- Levan molecular weight (oligosaccharides),
- Hydrolytic activity,
- Acceptor reaction efficiency

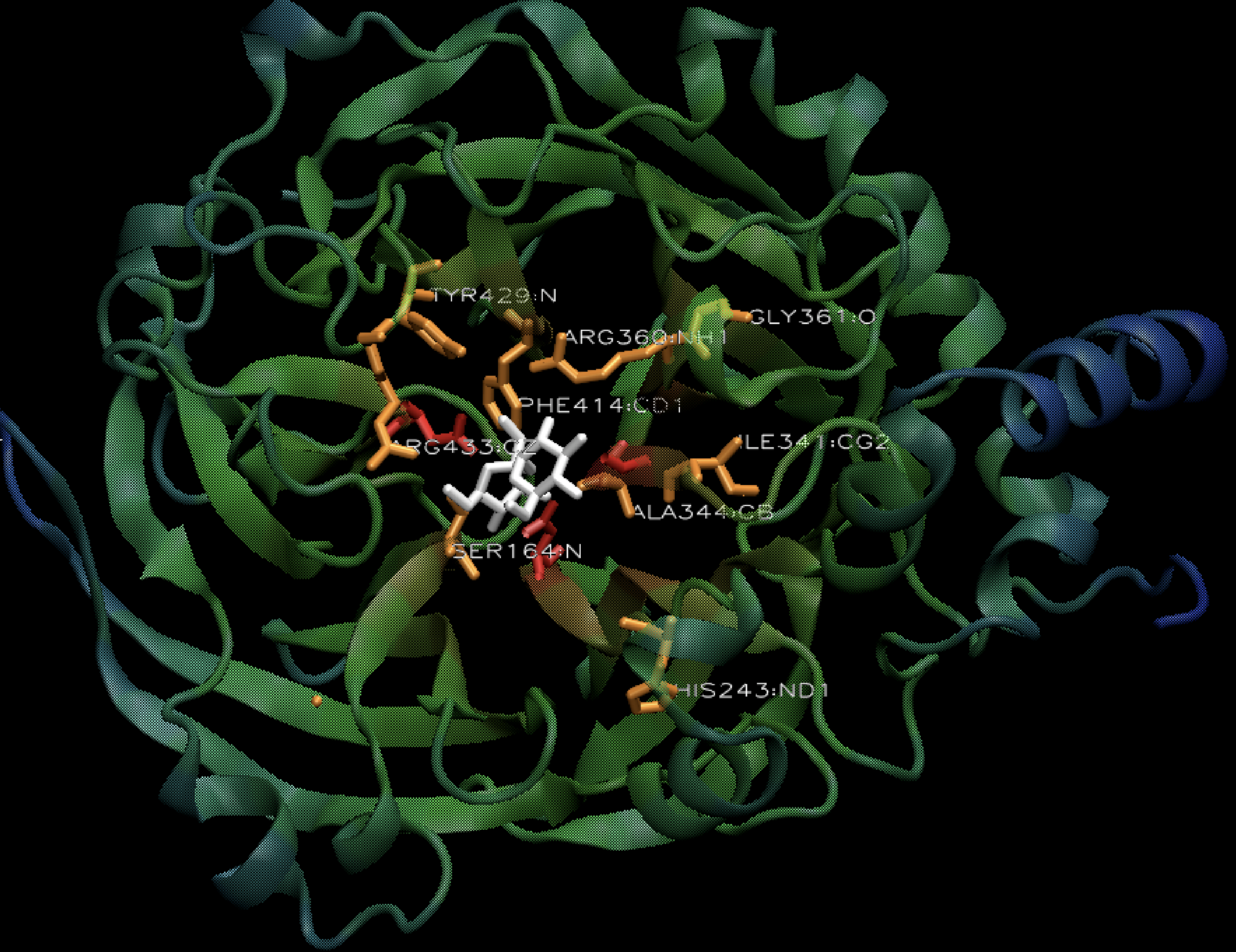
- Stability
- Temperature-activity profile
- pH-activity profile
- Activity in organic solventsx



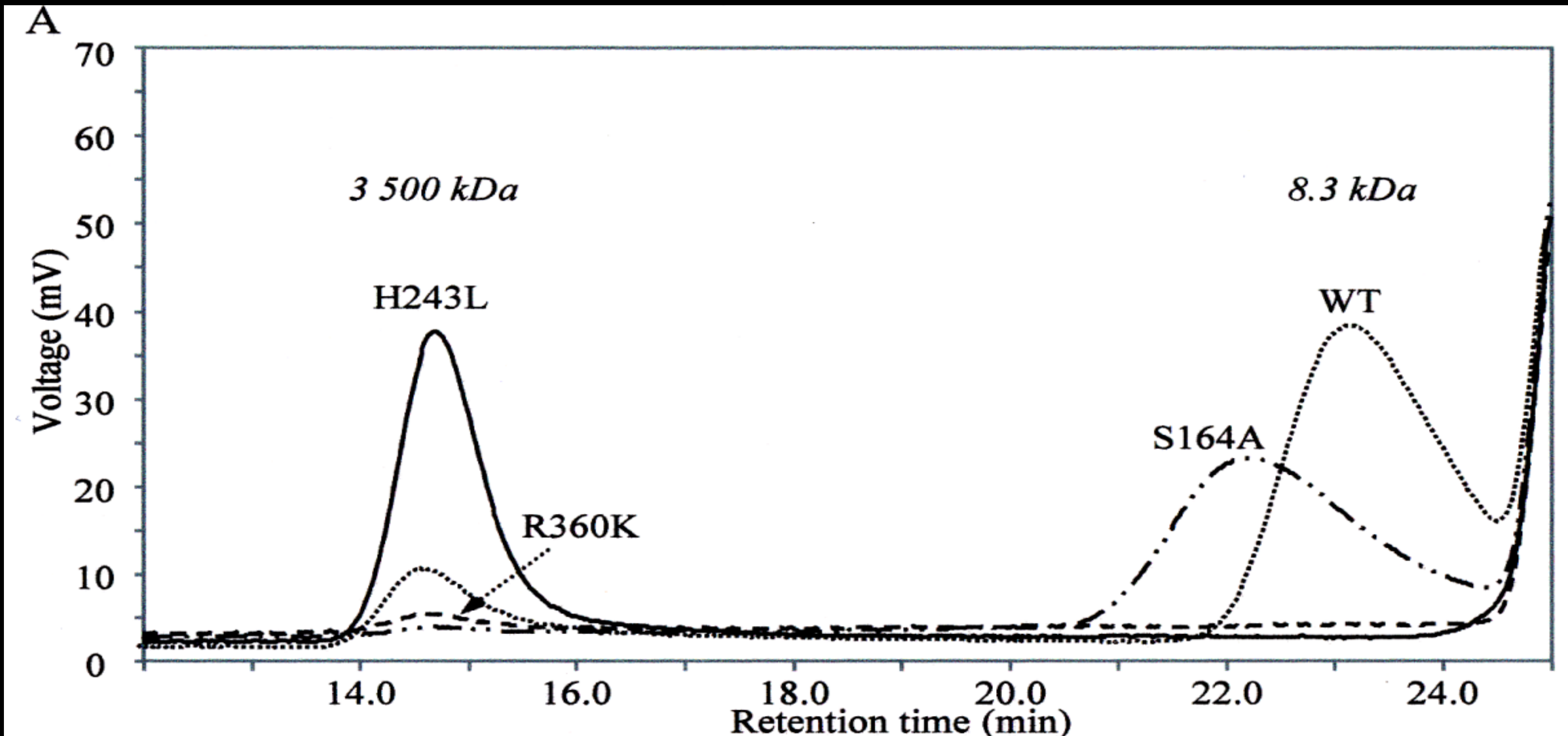
$$K_{m\,H2O} = \frac{k_{2\,H2O} + k_{-1}}{k_1}$$

$$K_{m\,T} = \frac{K_{2\,T} + k_{-1}}{k_1}$$

S164A/K/T
H243L
I341V
A344P
R360K/S
G361F
F414W
Y429/F/H/N
R433A



- Sequence alignment of 36 members of Family 68
- 6 semi-conserved regions found around the catalytic cavity
- 9 aa selected based on position of the lateral chain and distance from the glycosidic linkage (< 15 Å)
- Construction of mutants based on conserved aa in the selected position
- Expresison in E.coli BL 21 (PET 22)


A
70
60
50
40
30
20
10
0
Voltage (mV)
3 500 kDa
8.3 kDa
H243L
WT
S164A
R360K
14.0
16.0
18.0
20.0
22.0
24.0
Retention time (min)

β2-6 Lev-FOS as prebiotics

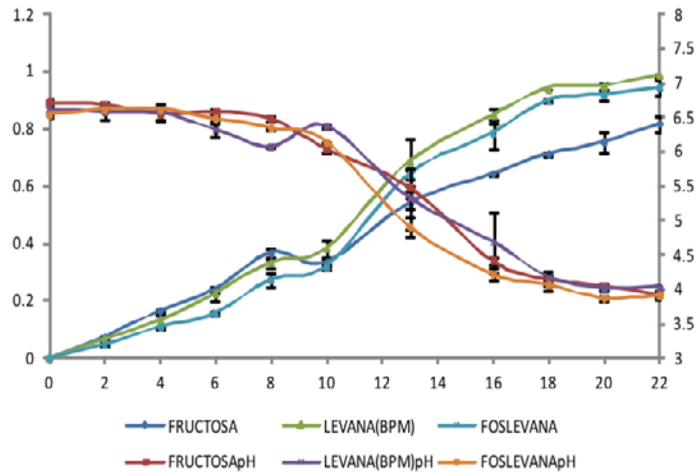


Fig.5 Cinéticas de crecimiento de *B. infantis* con diferentes fuentes de carbono, datos de Densidad óptica (660nm).

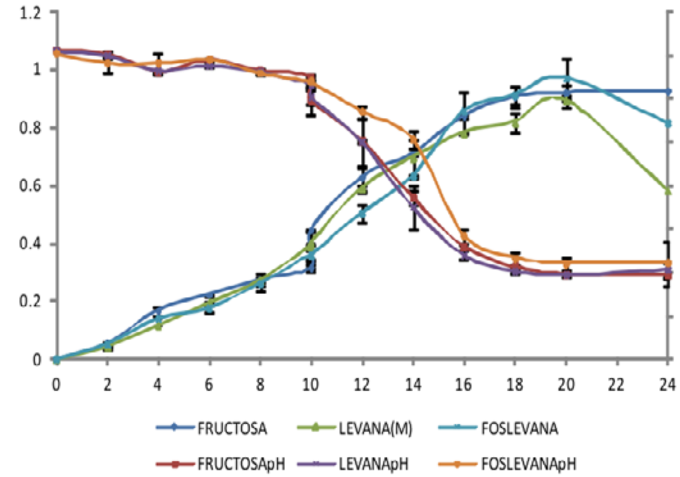


Fig.7 Cinéticas de crecimiento de *Lactobacillus paracasei* con diferentes fuentes de carbono, datos de Densidad óptica (660nm).

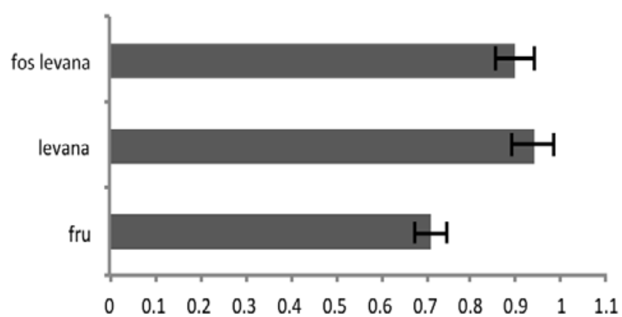


Fig.6 Densidades ópticas máximas (660nm), obtenidas por *B. infantis* con los tres diferentes sustratos (18h).

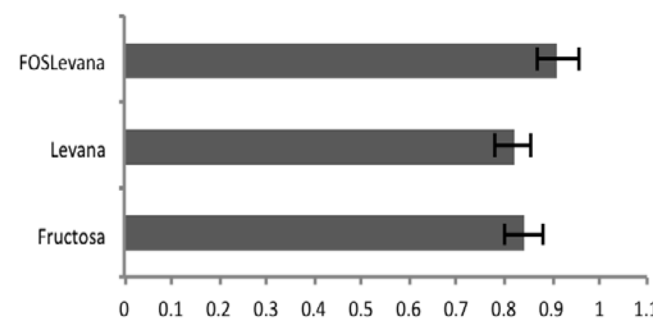
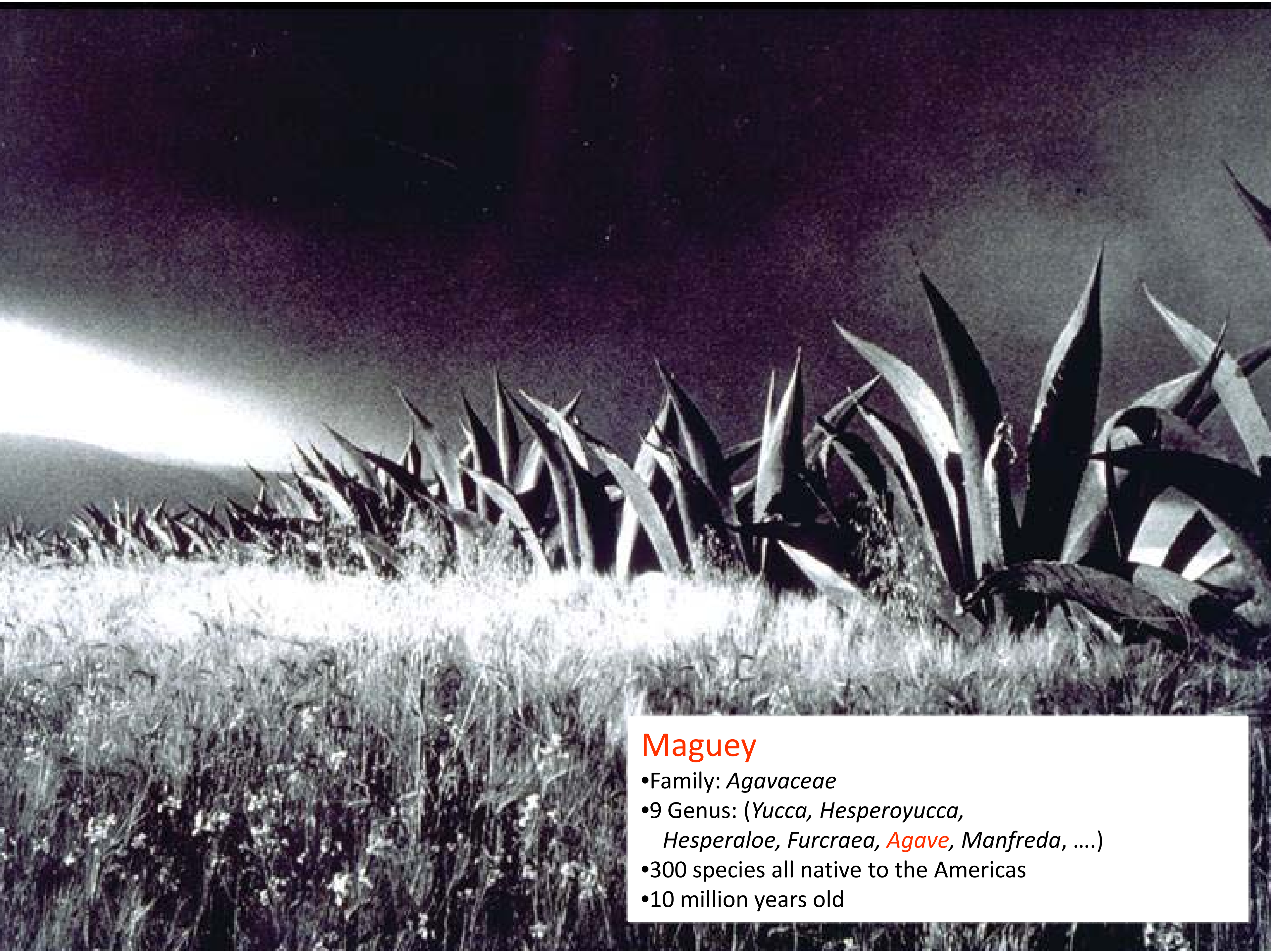


Fig.8 Densidades ópticas máximas (660nm), obtenidas por *Lactobacillus paracasei* con Fructosa (16 h), FOS de Levana y levana (18 h).



Maguey

- Family: *Agavaceae*
- 9 Genus: (*Yucca, Hesperoyucca, Hesperaloe, Furcraea, Agave, Manfreda*, ….)
- 300 species all native to the Americas
- 10 million years old

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Water-Soluble Carbohydrates and Fructan Structure Patterns from *Agave* and *Dasylirion* Species

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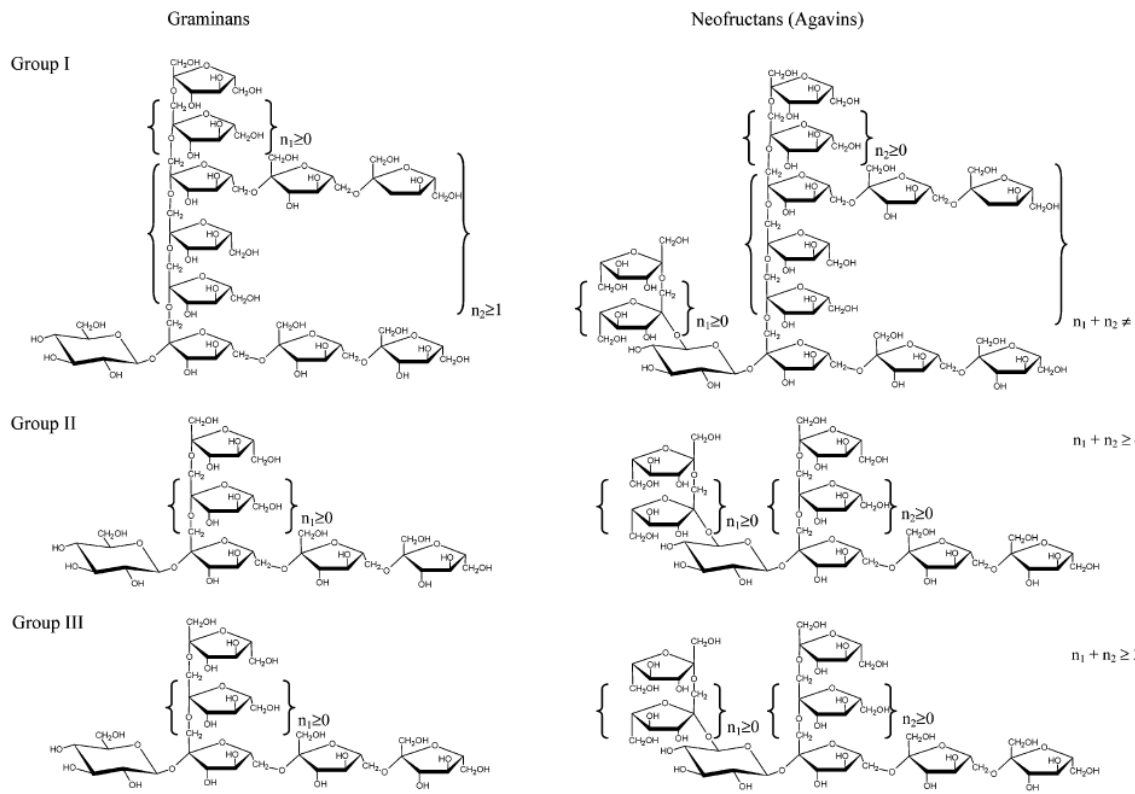
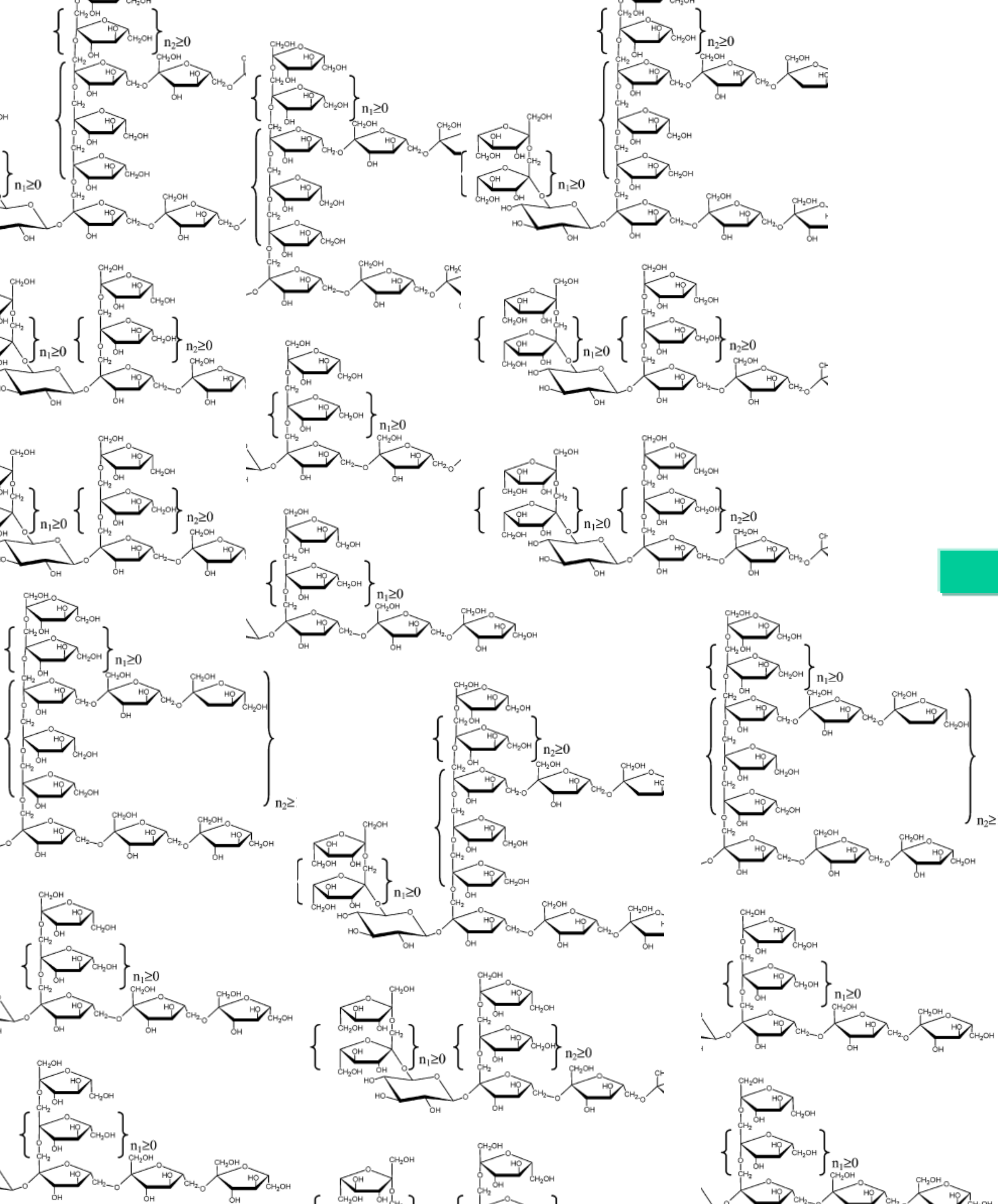
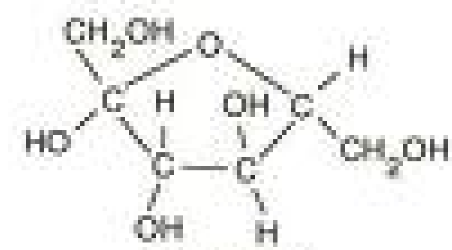


Figure 4. Proposed fructan structures for *Agave* and *Dasylirion* species. Molecular structures based on the three proposed groups, and two types of fructans within the groups (A for graminans and B for agavins).

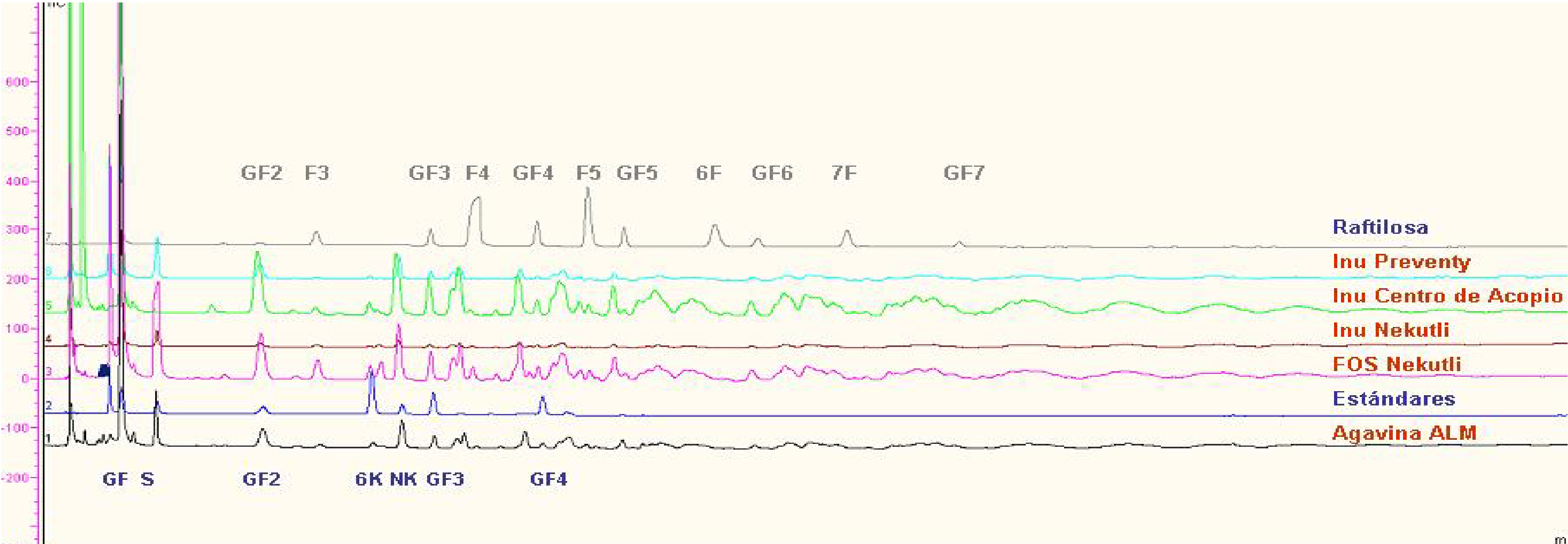


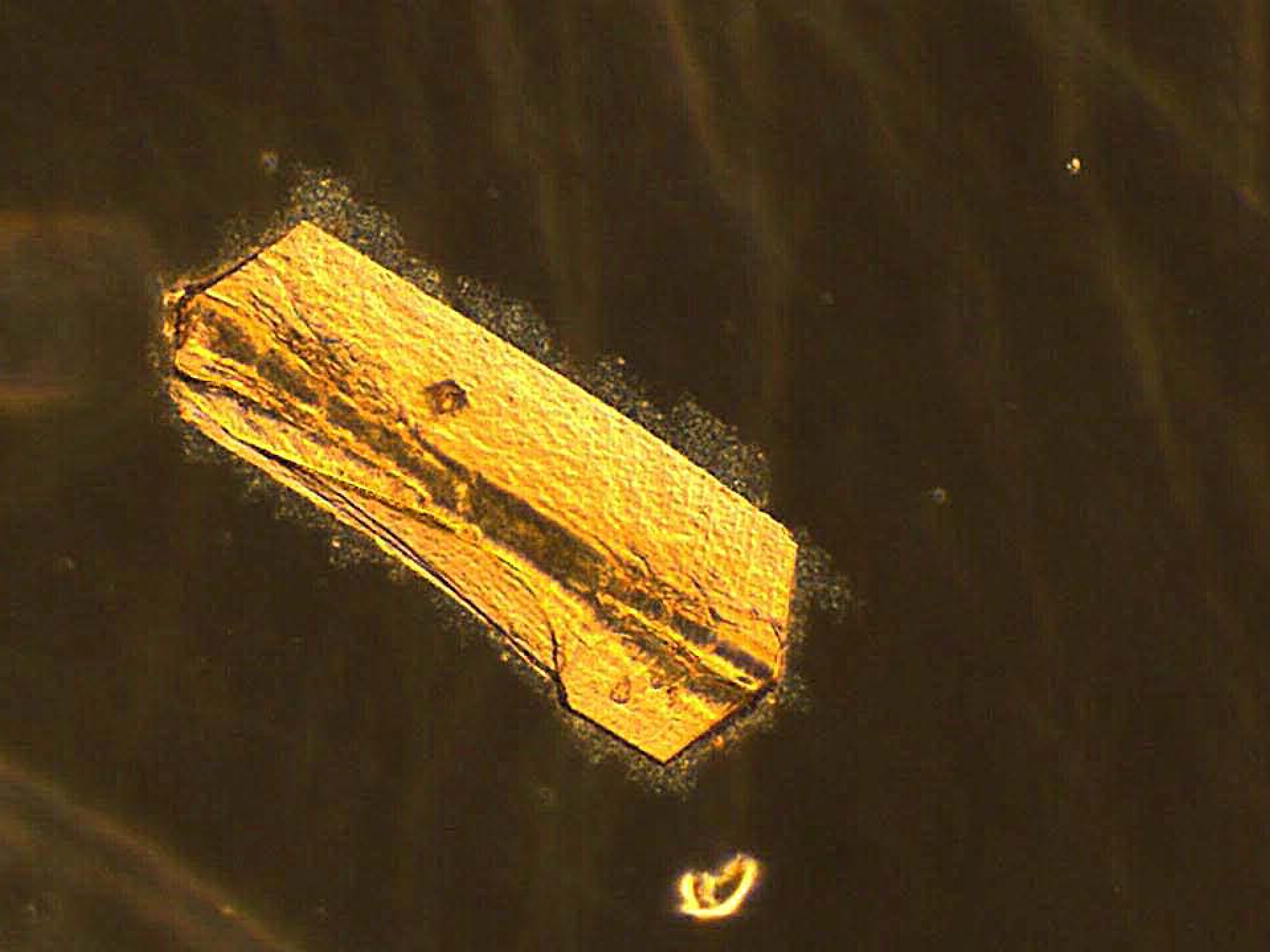

Naturaleve
Néctar de Agave Orgánico
100% Natural
REMEDIOS MÉXICO
MIEL DE AGAVE
Agave Nectar
ENDULZANTE NATURAL


CH2OH
O
H
HO
C
H
OH
C
CH2OH
C
C
OH
H

Fructose

Continuous extraction


600
500
400
300
200
100
0
-100
-200
GF2 F3 GF3 F4 GF4 F5 GF5 6F GF6 7F GF7
Raftilosa
Inu Preventy
Inu Centro de Acopio
Inu Nekutli
FOS Nekutli
Estándares
Agavina ALM
GF S GF2 6K NK GF3 GF4



Instituto de Biotecnología

Thank you for your attention