GENOMIC ADAPTATIONS OF DOMINANT SUGARCANE FERMENTING YEAST STRAINS

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WFCC ICCC-12 Conference 2010
WORLD FEDERATION FOR CULTURE COLLECTIONS
Biological Resource Centers: gateway to biodiversity and services for innovation in biotechnology

Sustainability and Energy

PERSPECTIVE

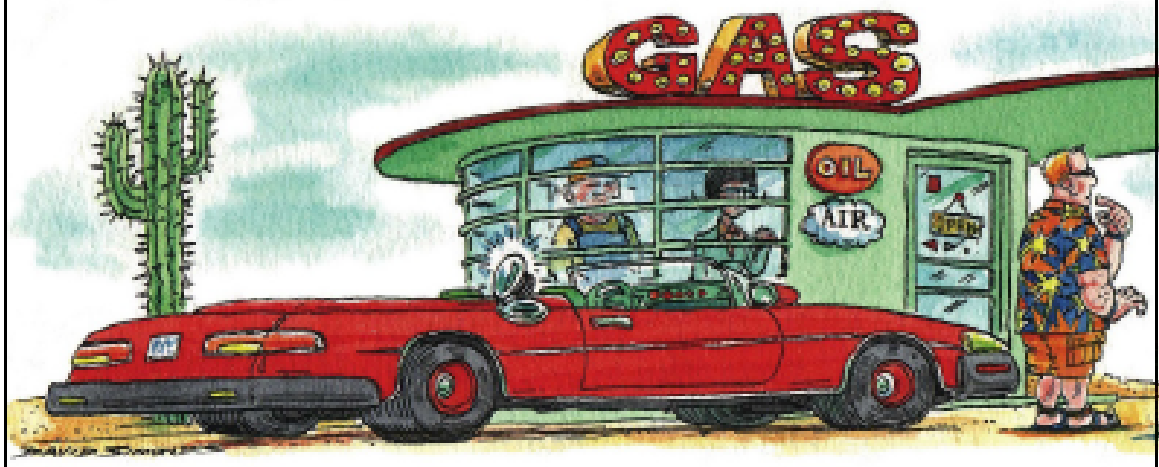
Ethanol for a Sustainable Energy Future

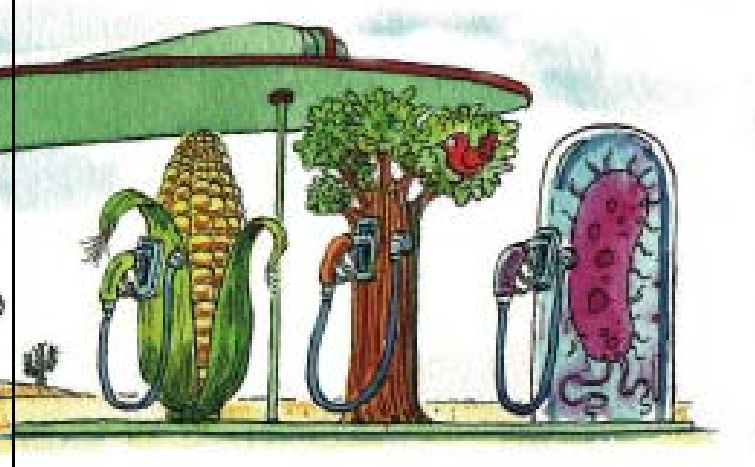
José Goldemberg*

9 FEBRUARY 2007 VOL 315 SCIENCE www.sciencemag.org

Drink the best and drive the rest

Brazil's sugar-cane ethanol industry is the world's best and able to get better, says **Emma Marris**.



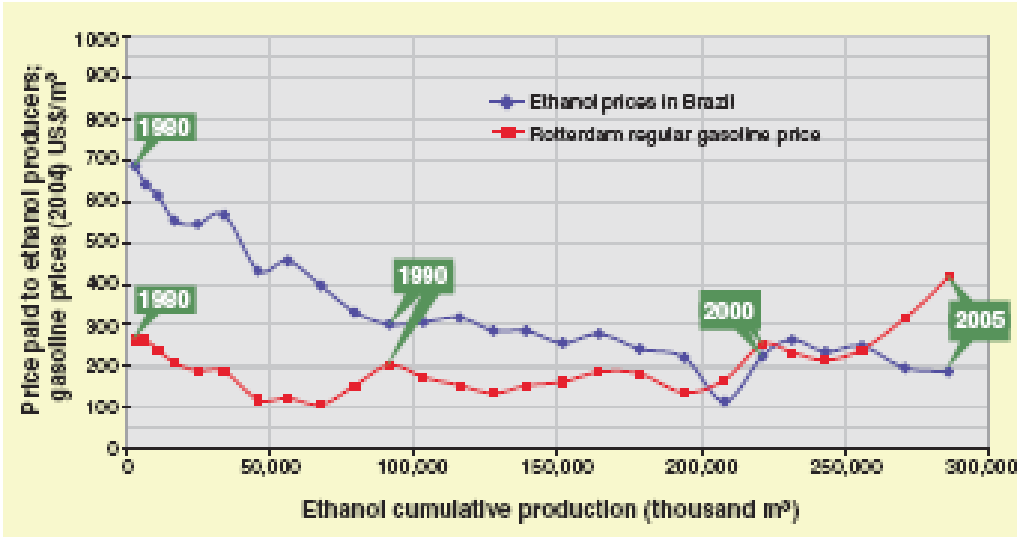


Cleaning up: Brazil's use of sugar cane, here being washed for refining, significantly reduces CO_2 emissions.

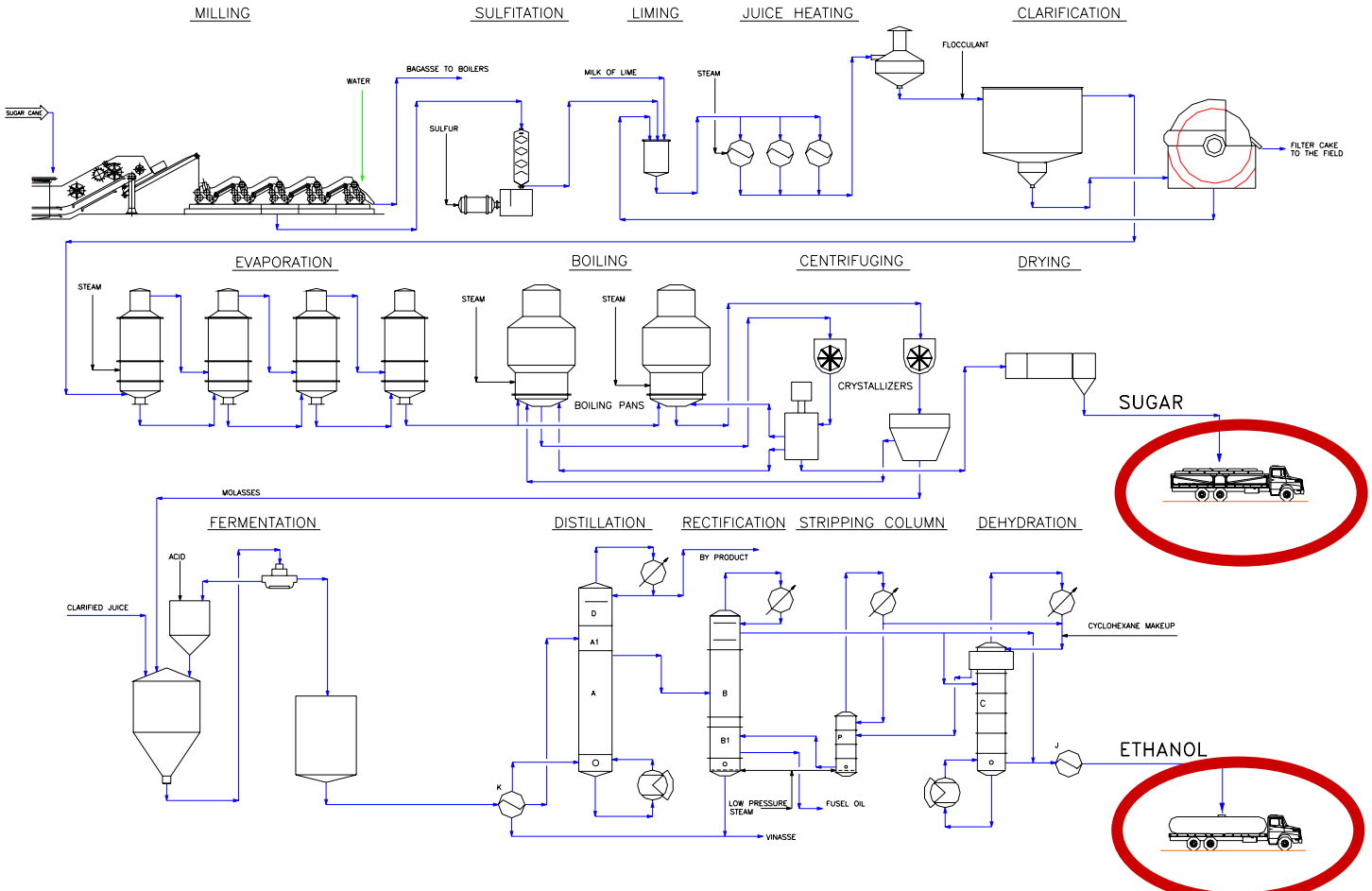
BIOFUELLING THE FUTURE

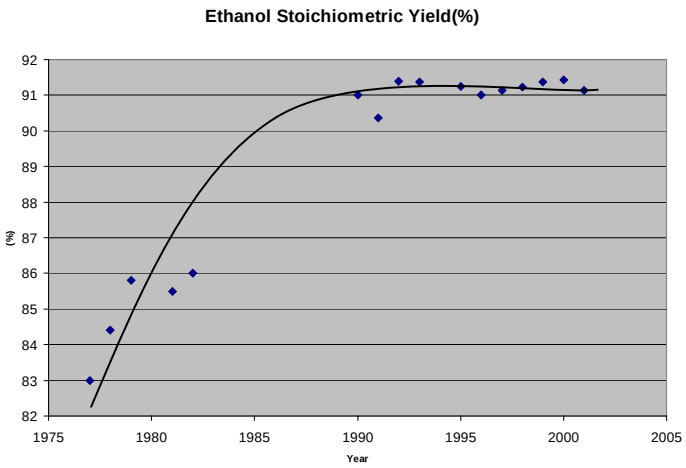
BRAZIL

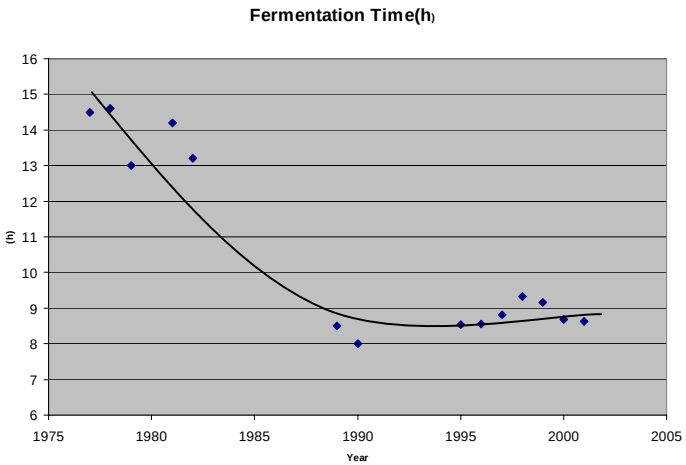
~24 billion L fuel ethanol
~1(?) billion L of Cachaça



FLOW DIAGRAM – SUGAR AND ETHANOL







Main parameters:

[sugar]:	180-200 g/L
Final ethanol content:	9ºGl (%vol)
Final yeast concentration:	13% (~10^9 cells/ml)
Fermentation time:	6-11 h

Fed-batch or continuous multistage with cell recycle

Total fermenter capacity:	3000 m^3
Yield (stoichiometric):	91%
Temperature:	34-36ºC

(~March……. into…… ~October)

Yeast ?

Baker's Yeast

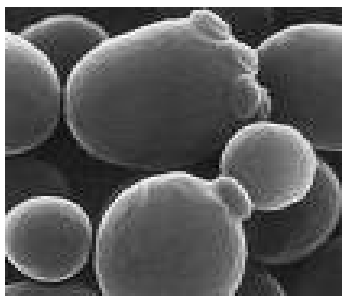
Molasses

Cane juice

Lactobacillus
Acetobacter
Saccharomyces
Dekkera/Brettanomyces
Candida
Schizosaccharomyces
Pichia
Kloeckera
Kluyveromyces
Torulopsis

Selected Yeasts

(*Saccharomyces cerevisiae*)



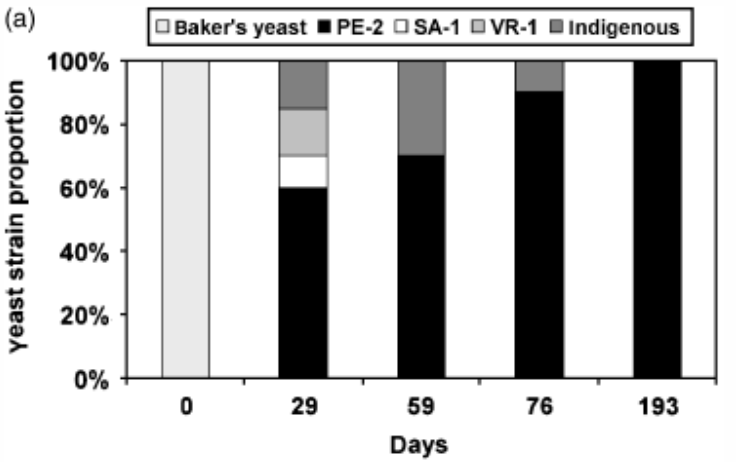
FEMS Yeast Res ■ (2008) 1–9

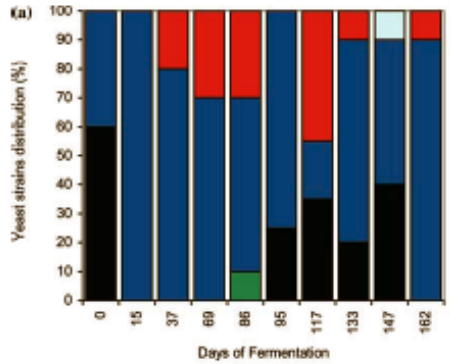
RESEARCH ARTICLE

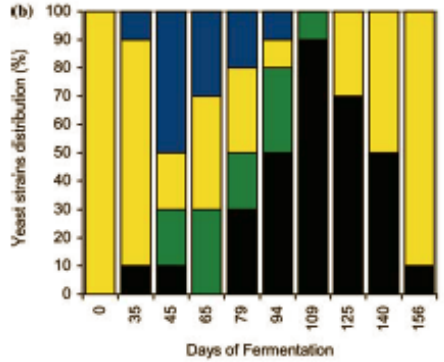
Yeast selection for fuel ethanol production in Brazil

Luiz C. Basso[1], Henrique V. de Amorim[2], Antonio J. de Oliveira[2] & Mario L. Lopes[2]

[1]Biological Science Department, Escola Superior de Agricultura Luiz de Queiroz, USP, Piracicaba, SP, Brazil; and [2]Fermentec, Piracicaba, SP, Brazil





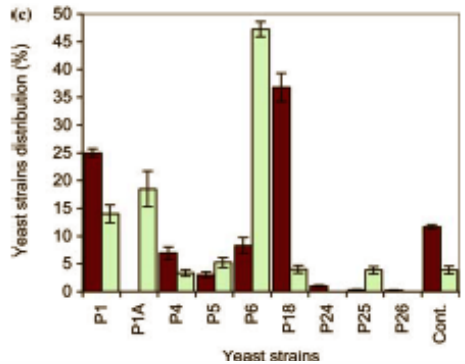


Use of selected indigenous *Saccharomyces cerevisiae* strains for the production of the traditional cachaça in Brazil

F.C.O. Gomes[1,2], C.L.C. Silva[1], M.M. Marini[1], E.S. Oliveira[3] and C.A. Rosa[1]

1 Departamento de Microbiologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil
2 Departamento de Química, Centro Federal de Ensino Tecnológico, Belo Horizonte, Minas Gerais, Brazil
3 Departamento de Alimentos, Faculdade de Farmácia, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

Journal of Applied Microbiology 103 (2007) 2438–2447



Antonie van Leeuwenhoek (2005) 88:13–23
DOI 10.1007/s10482-004-7283-8

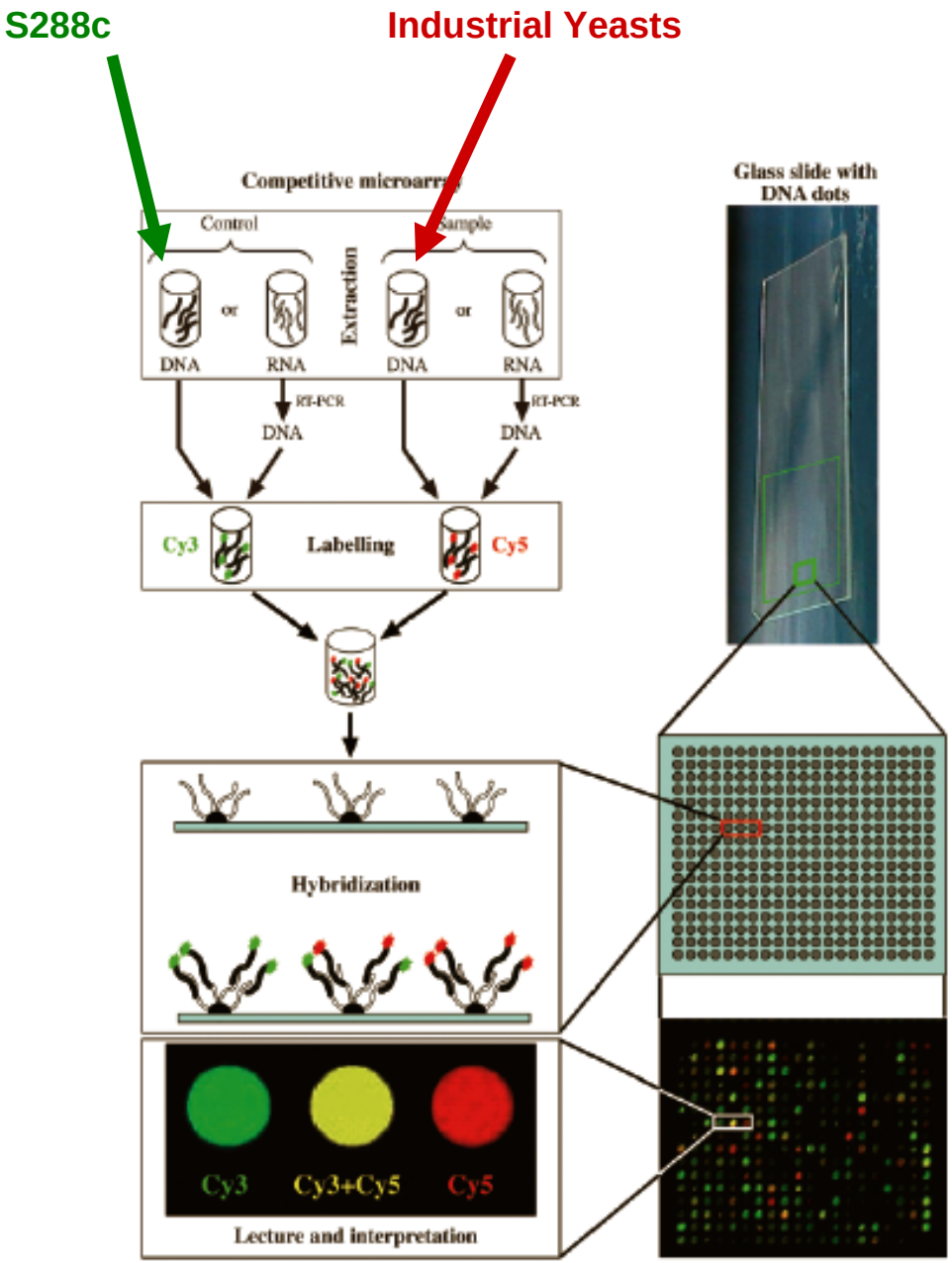
Yeast population dynamics of industrial fuel-ethanol fermentation process assessed by PCR-fingerprinting

Eurípedes Alves da Silva-Filho[1,2], Scheila Karina Brito dos Santos[3], Alecsandra do Monte Resende[3], José Otamar Falcão de Morais[4], Marcos Antonio de Morais Jr[1,3,*] and Diogo Ardaillon Simões[1,5]

Fermentec:
CAT-1; PE-2; VR-1
Copersucar: **>10 billion L ethanol / year**
BG; SA

UFPE-135; UFPE-379

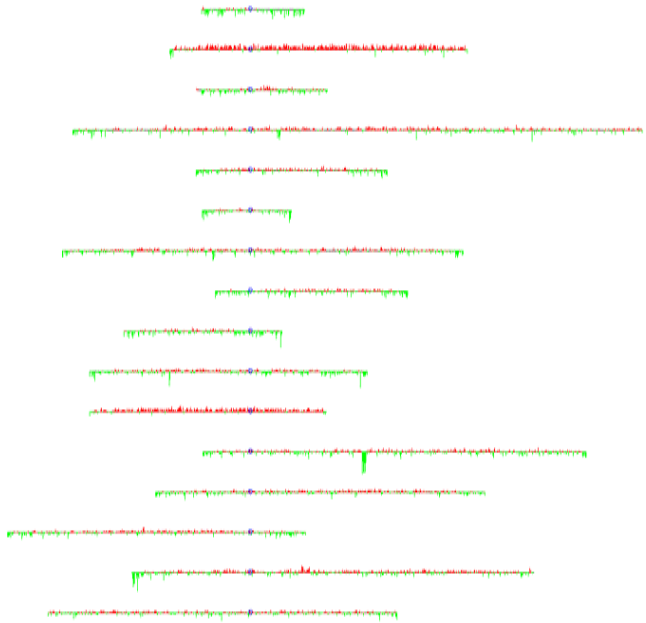
UFMG (cachaça):
829; 905; 1007; 2097; 2439



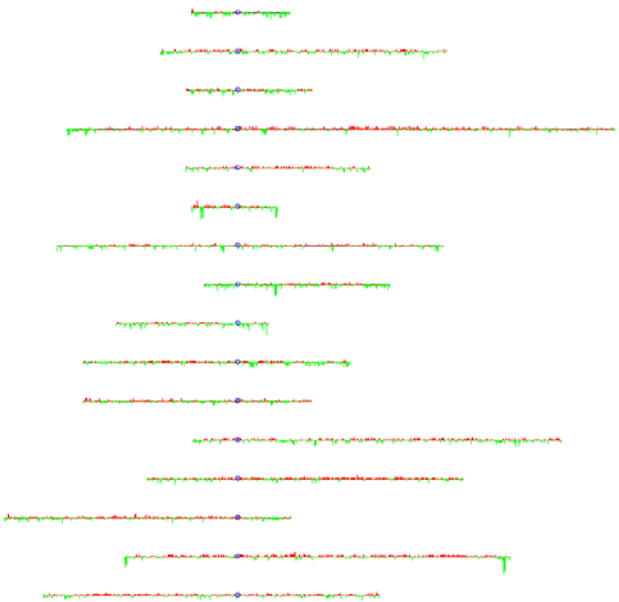
array-CGH
(microarray Comprehensive Genomic Hybridization)

Microarray Karyotyping

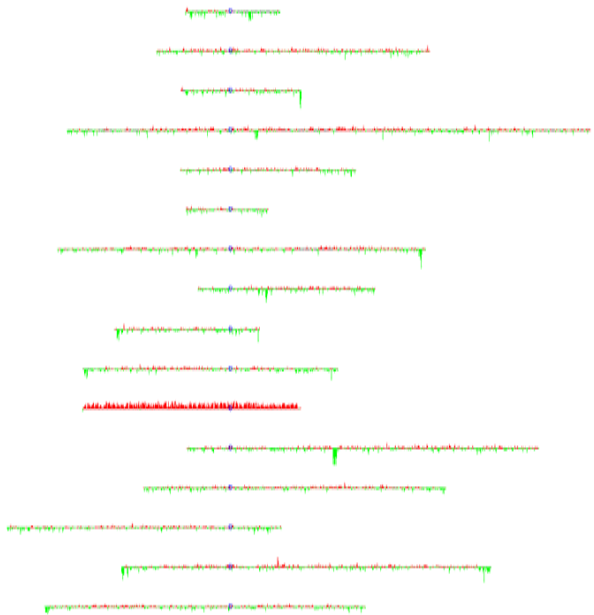
PE-2



UFMG905

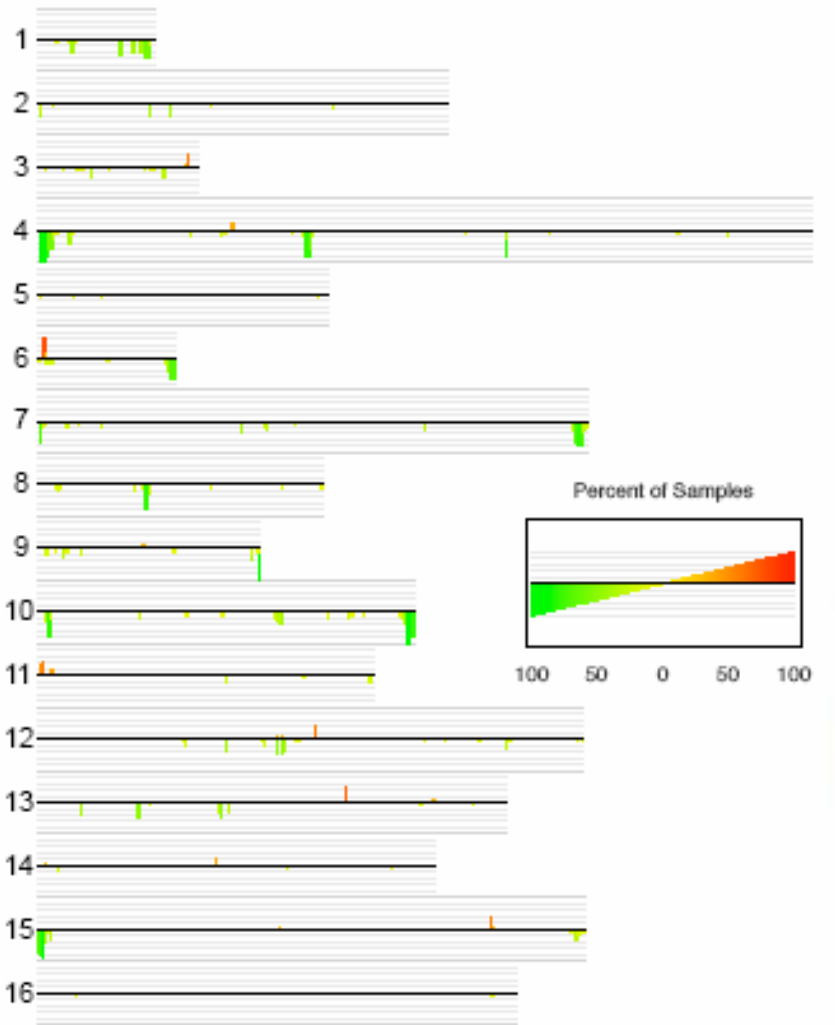


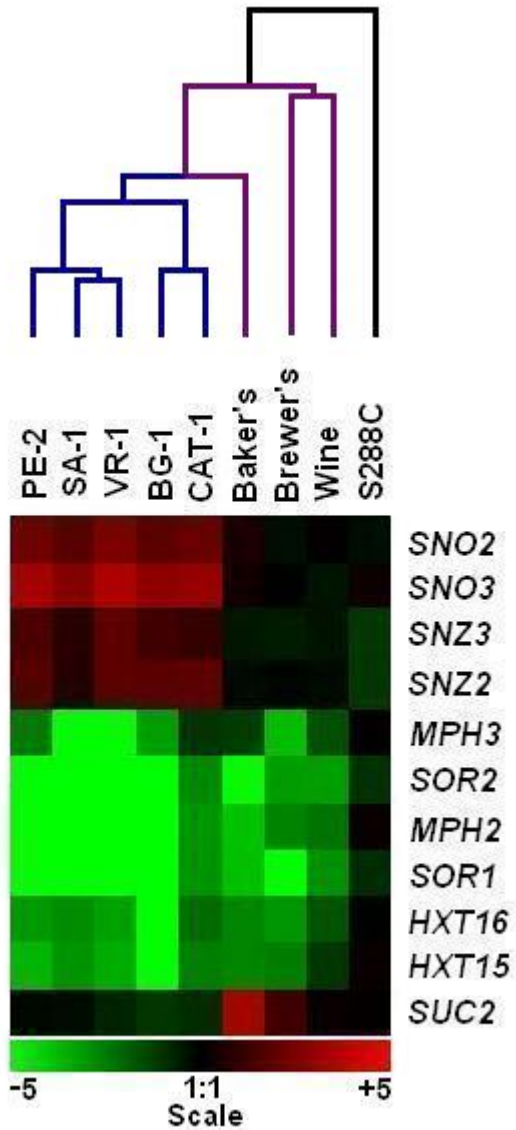
CAT-1

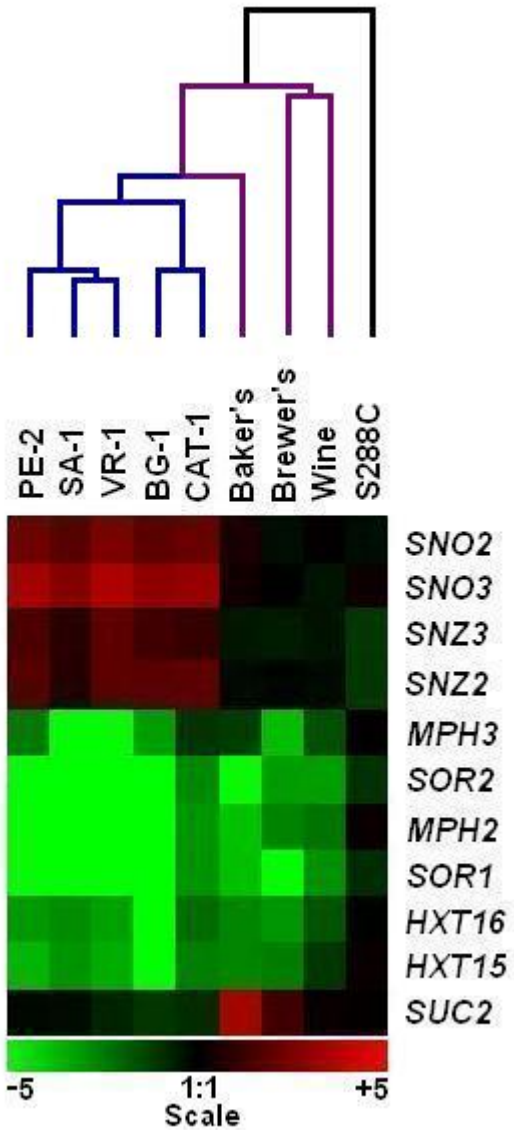


UFMG829

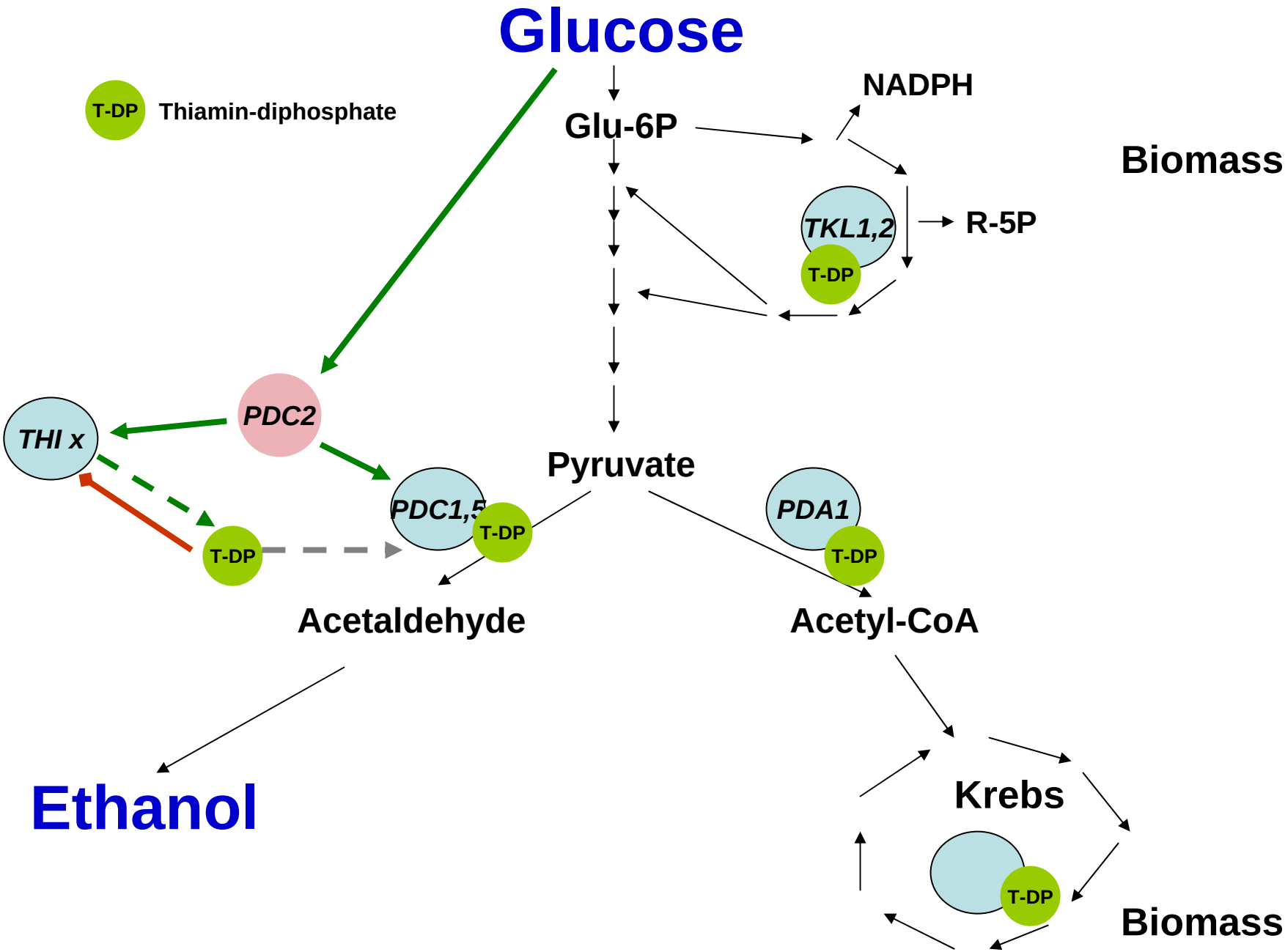
CLAC Caryoscope:

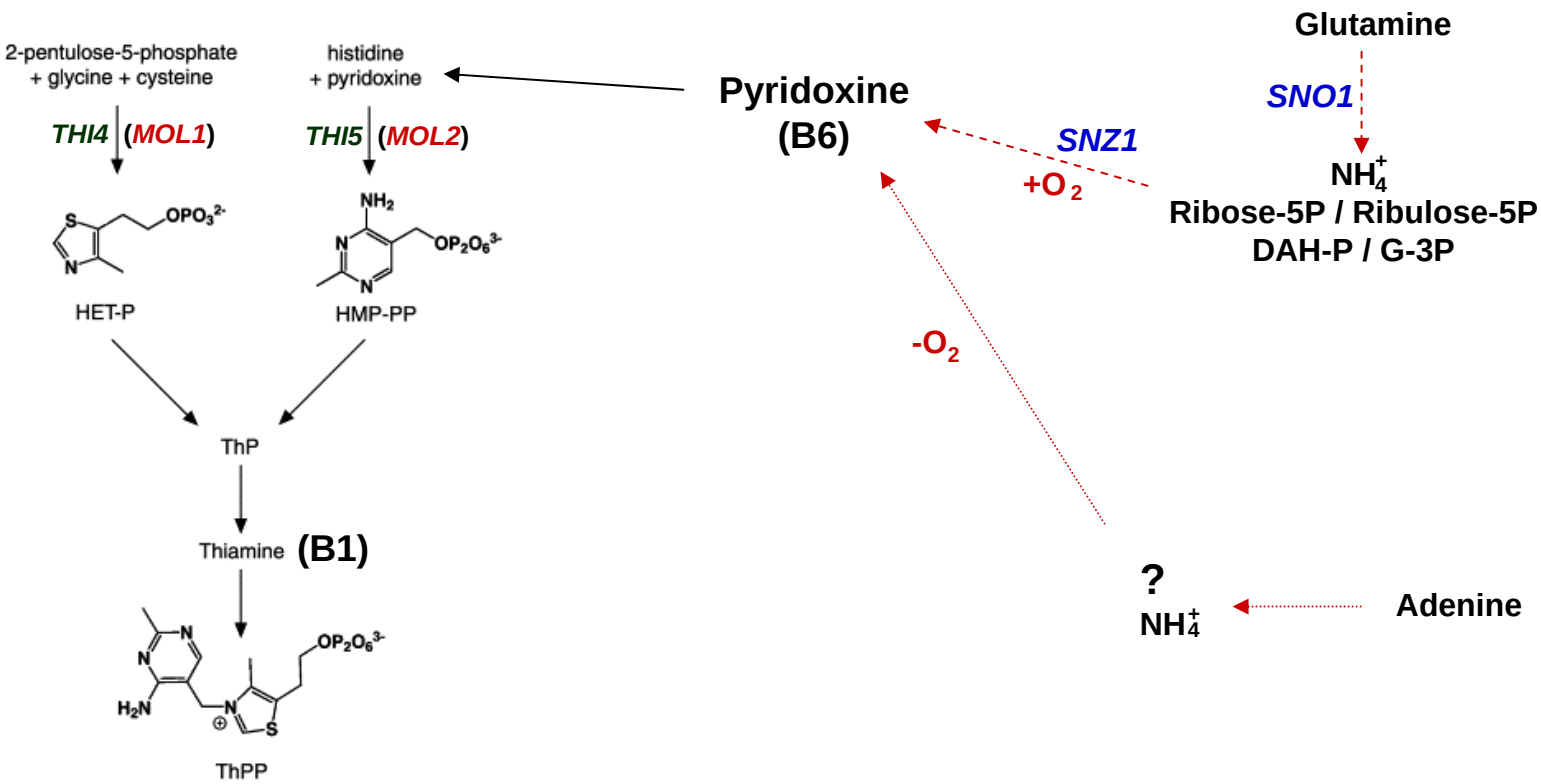






Pyridoxine (& Thiamin) biosynthesis

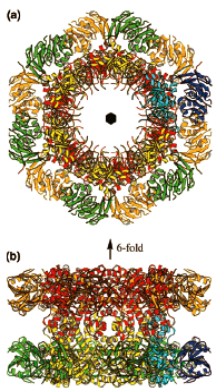

Glucose
T-DP
Thiamin-diphosphate
NADPH
Glu-6P
Biomass
TKL1,2
T-DP
R-5P
PDC2
THI x
Pyruvate
PDC1,5
T-DP
T-DP
PDA1
T-DP
Acetaldehyde
Acetyl-CoA
Ethanol
Krebs
T-DP
Biomass

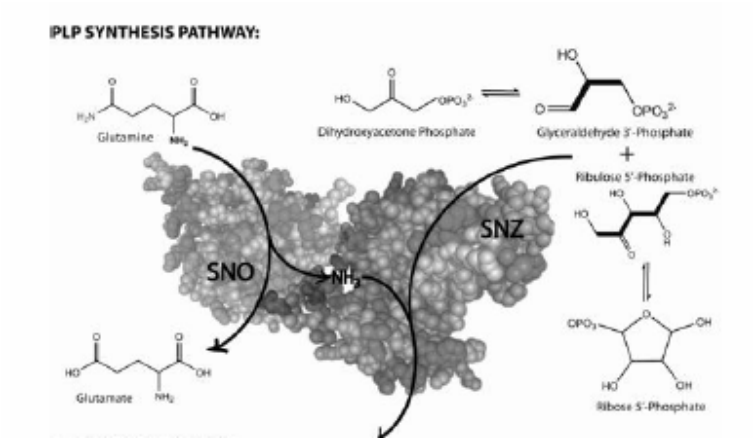

2-pentulose-5-phosphate
+ glycine + cysteine
histidine
+ pyridoxine
THI4 (MOL1)
THI5 (MOL2)
HET-P
HMP-PP
ThP
Thiamine (B1)
ThPP
Pyridoxine
(B6)
SNZ1
+O2
-O2
Glutamine
SNO1
NH4+
Ribose-5P / Ribulose-5P
DAH-P / G-3P
?
NH4+
Adenine

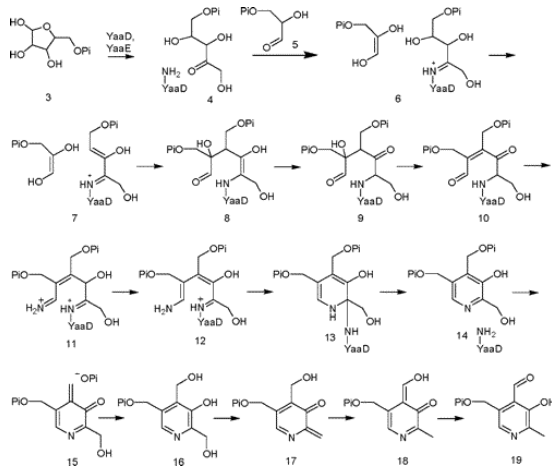
SNZ1	**~80%**	***SNZ2 / SNZ3***
SNO1	**~72%**	***SNO2 / SNO3***

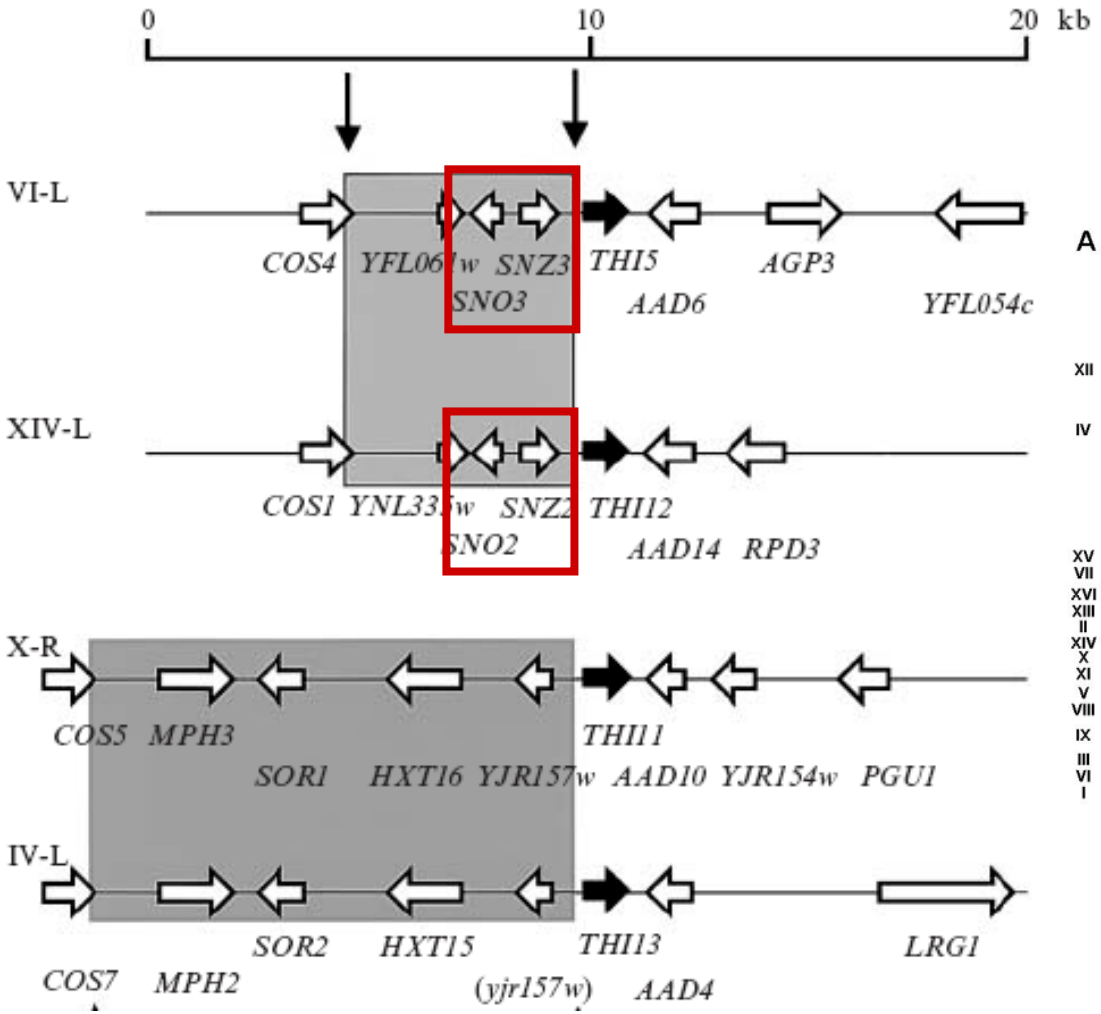
diauxic shift
stationary phase

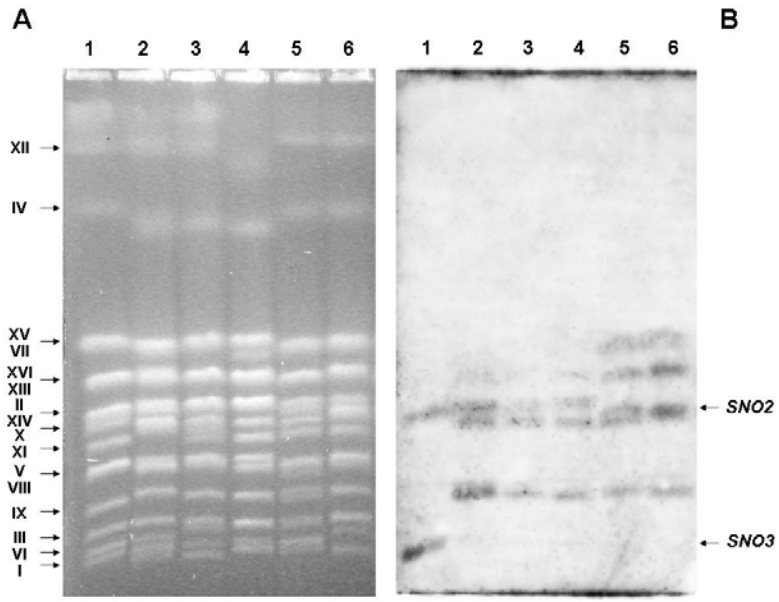
logarithmic (fermentative) phase
repressed by thiamin !

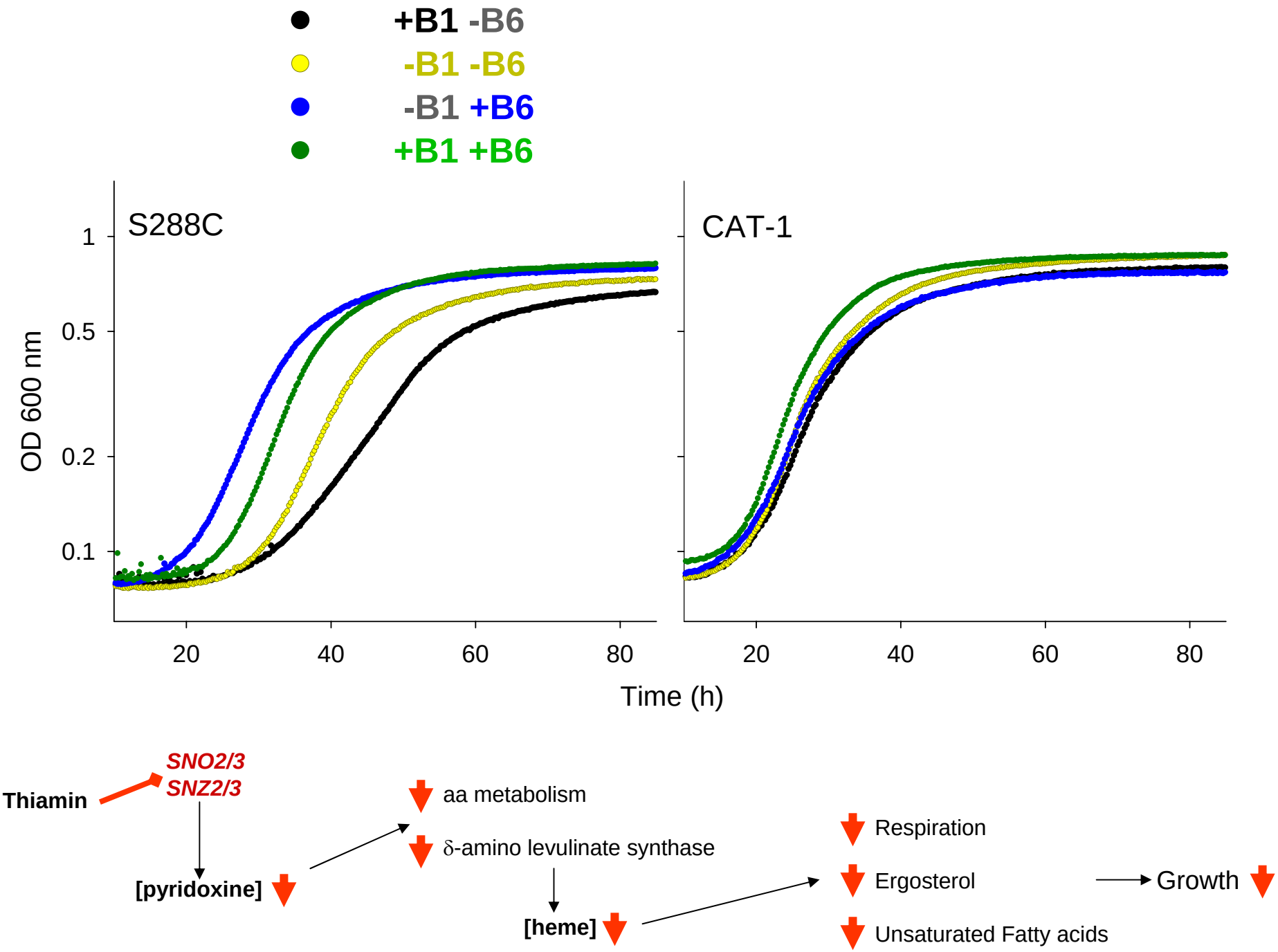


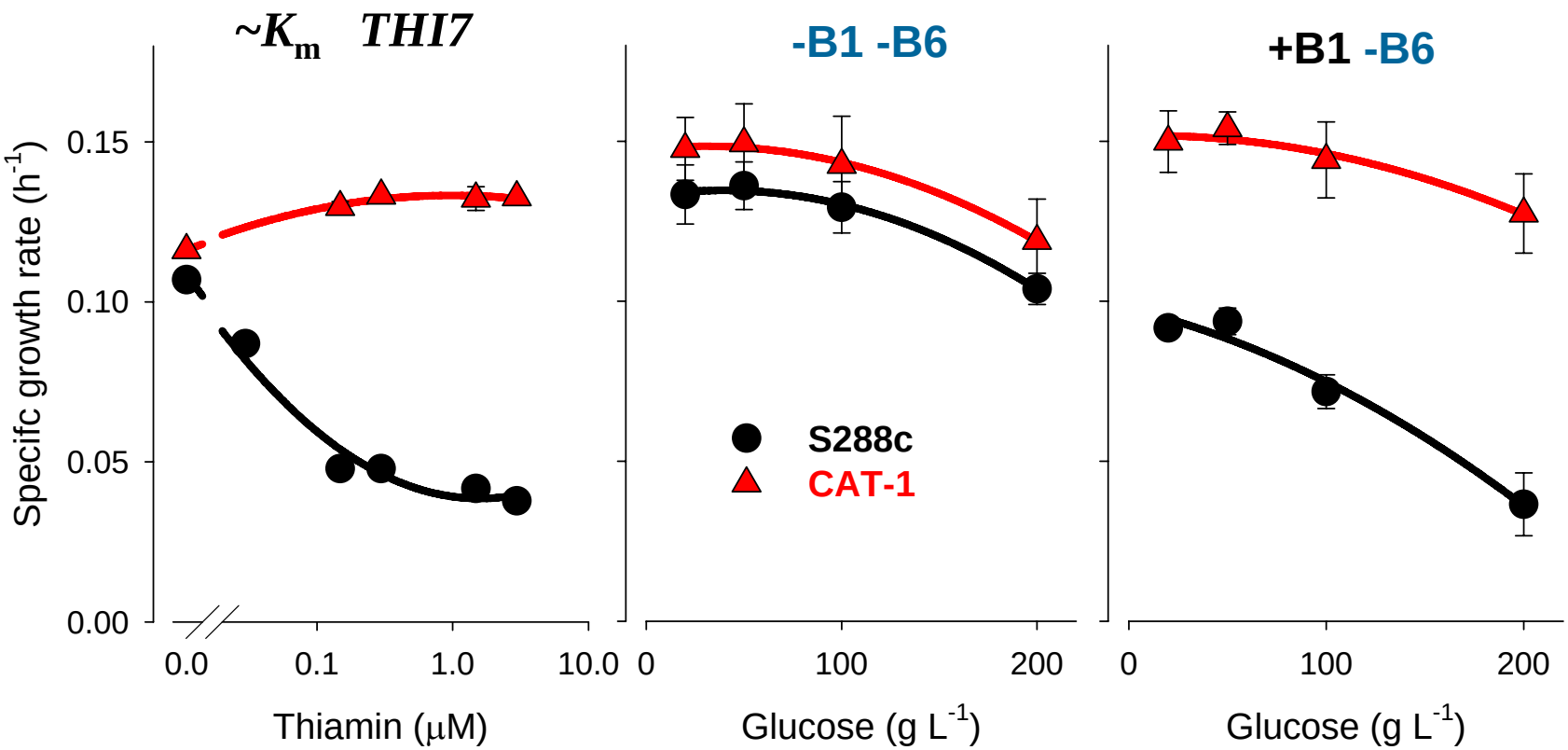





0
10
20 kb
VI-L
COS4 YFL061w SNZ3 THI5 AGP3
SNO3 AAD6 YFL054c
XIV-L
COS1 YNL333w SNZ2 THI12
SNO2 AAD14 RPD3
X-R
COS5 MPH3 THI11
SOR1 HXT16 YJR157w AAD10 YJR154w PGU1
IV-L
SOR2 HXT15 THI13 LRG1
COS7 MPH2 (yjr157w) AAD4

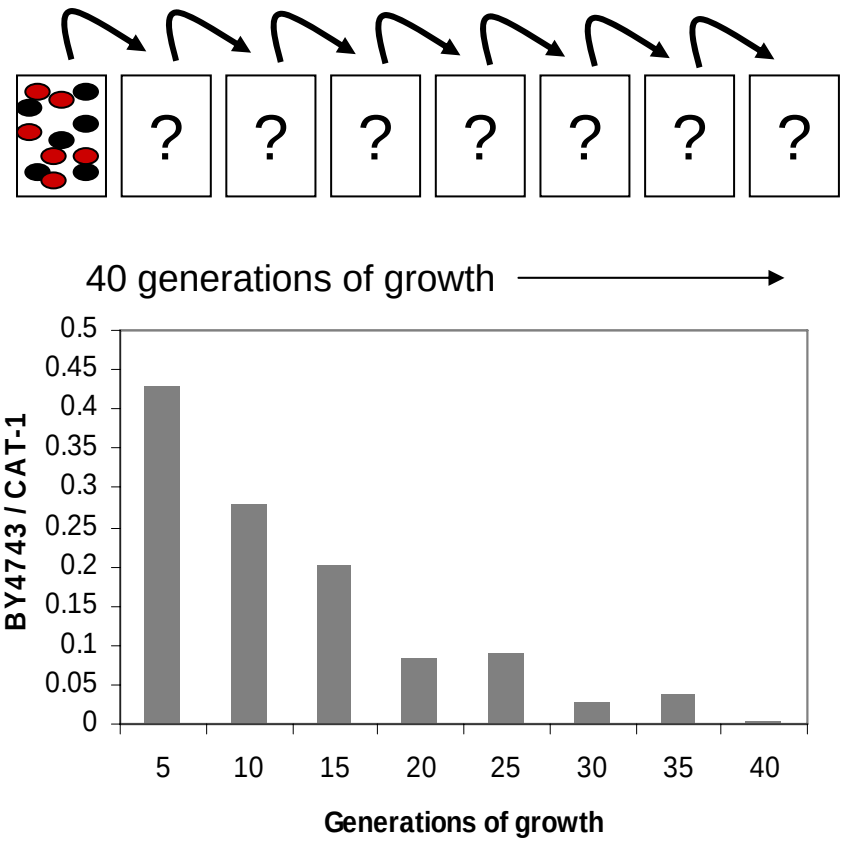

A
B
1 2 3 4 5 6
1 2 3 4 5 6
XII
IV
XV
VII
XVI
XIII
II
XIV
X
XI
V
VIII
IX
III
VI
I
SNO2
SNO3

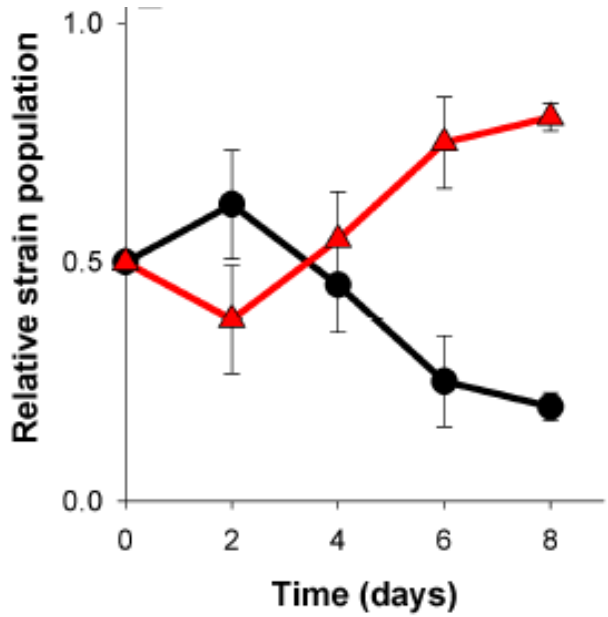

+B1 -B6
-B1 -B6
-B1 +B6
+B1 +B6
S288C
CAT-1
OD 600 nm
1
0.5
0.2
0.1
20
40
60
80
Time (h)
Thiamin
SNO2/3
SNZ2/3
[pyridoxine]
aa metabolism
δ-amino levulinate synthase
[heme]
Respiration
Ergosterol
Unsaturated Fatty acids
Growth

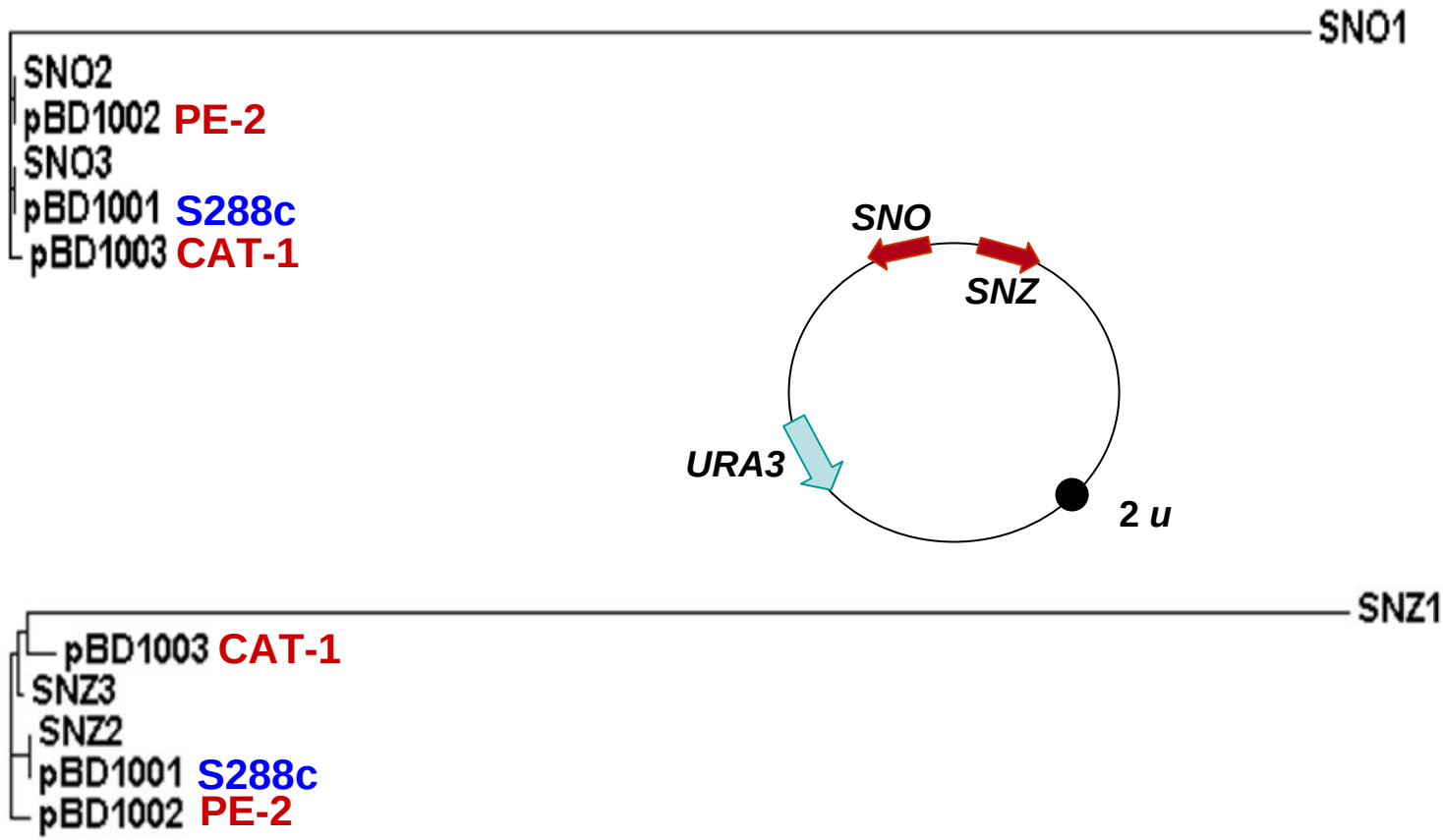


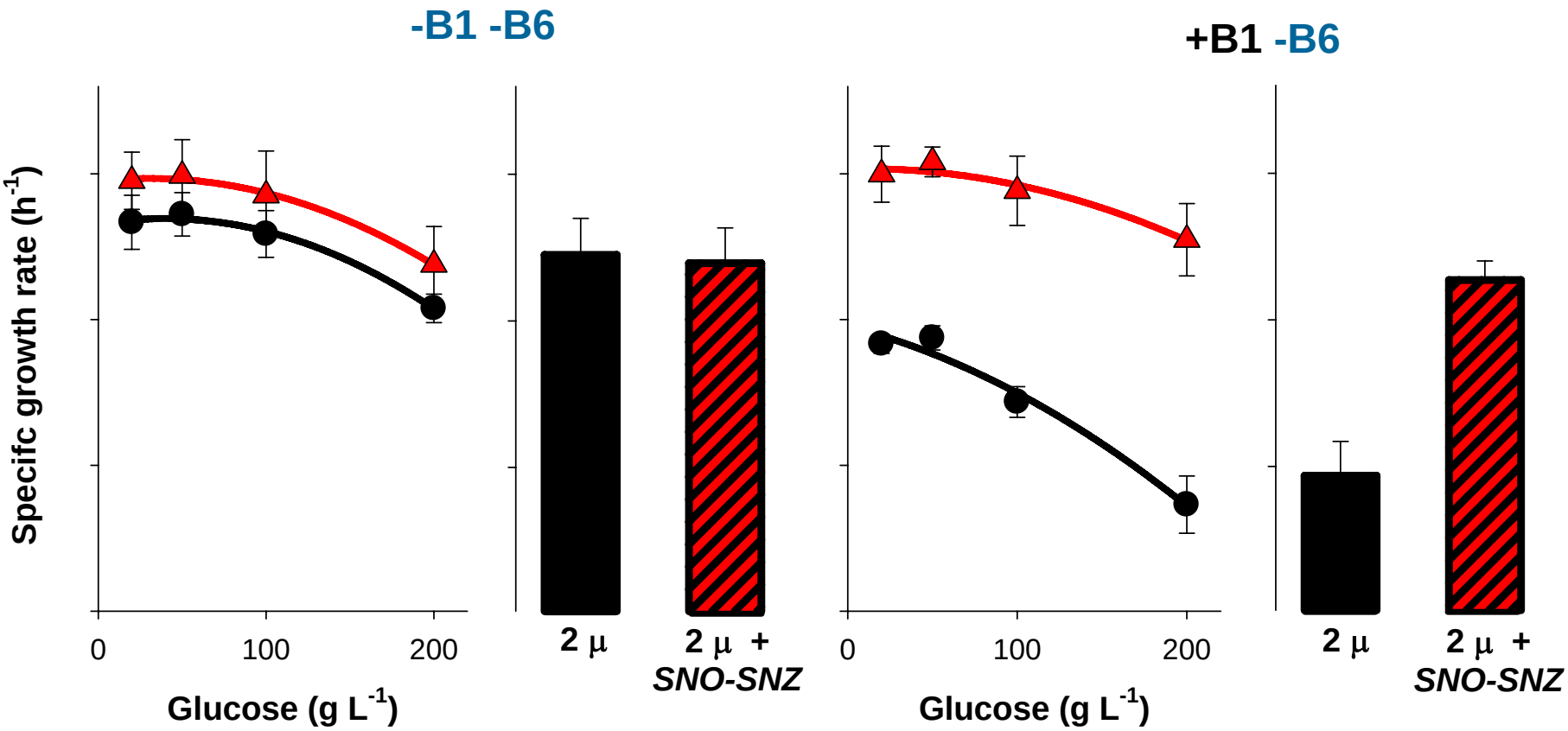
A phenotype important at high [sugar]

Direct competition






SNO1
SNO2
pBD1002 PE-2
SNO3
pBD1001 S288c
pBD1003 CAT-1
SNO
SNZ
URA3
2 u
SNZ1
pBD1003 CAT-1
SNZ3
SNZ2
pBD1001 S288c
pBD1002 PE-2

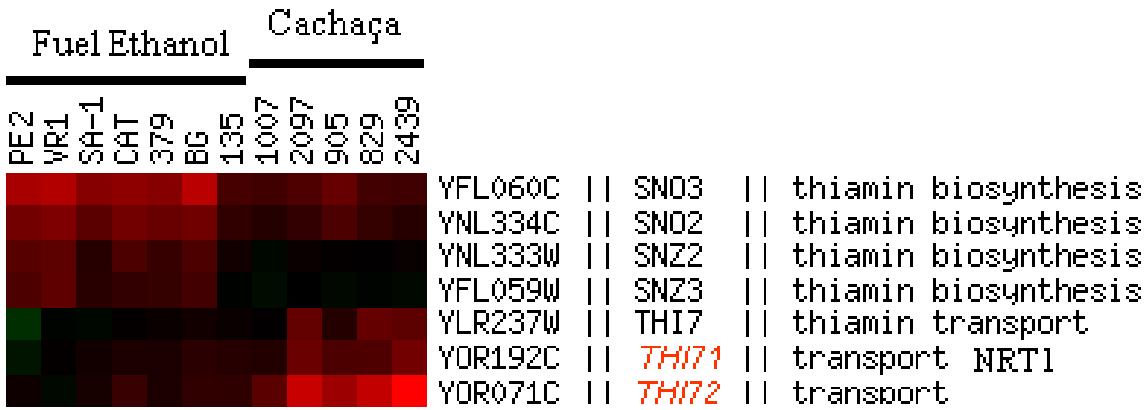


CSH PRESS GENOME RESEARCH

Industrial fuel ethanol yeasts contain adaptive copy number changes in genes involved in vitamin B1 and B6 biosynthesis

Boris U. Stambuk, Barbara Dunn, Sergio L. Alves, Jr, et al.

Genome Res. 2009 19: 2271-2278 originally published online November 6, 2009



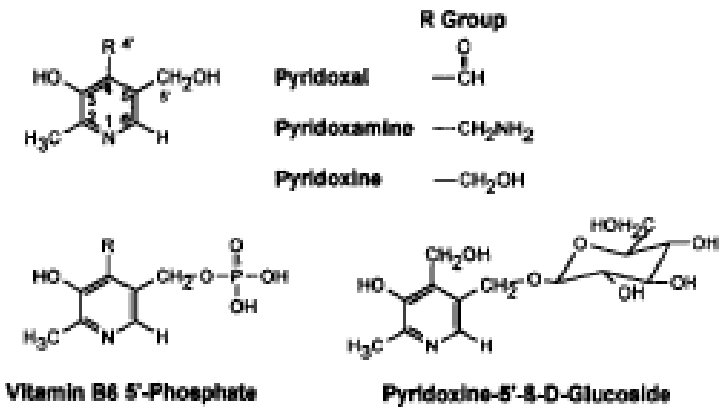
Sugar cane:

Thiamin ?

Pyridoxine ?

Cane juice ?

Molasses ?

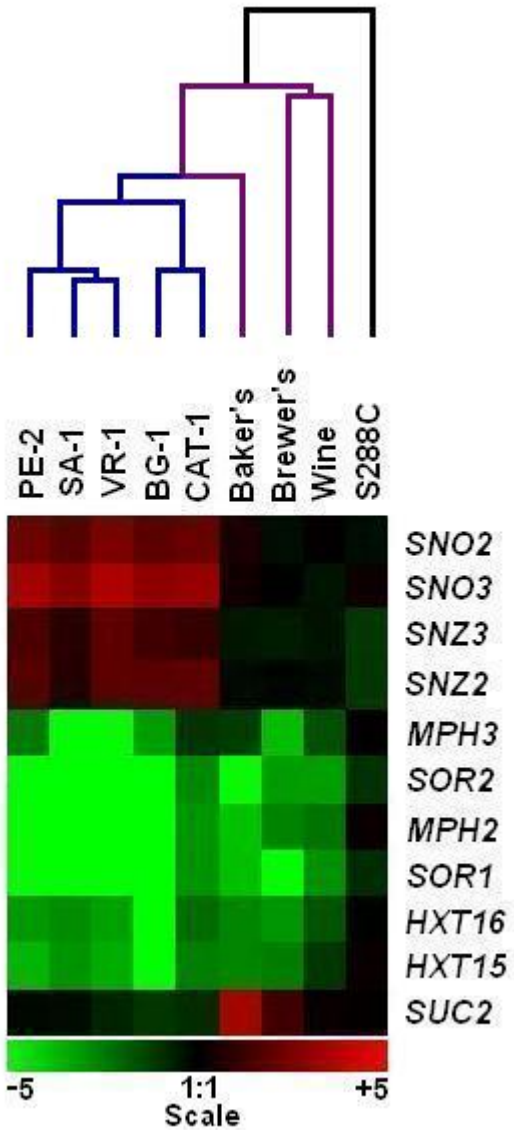


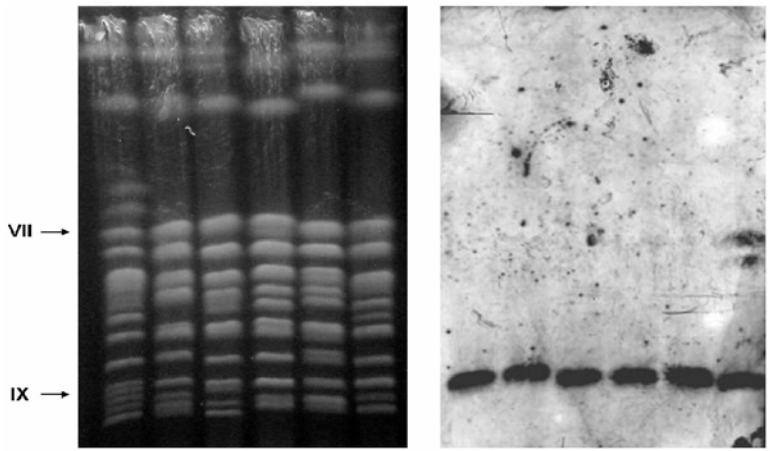
Bioavailability ?

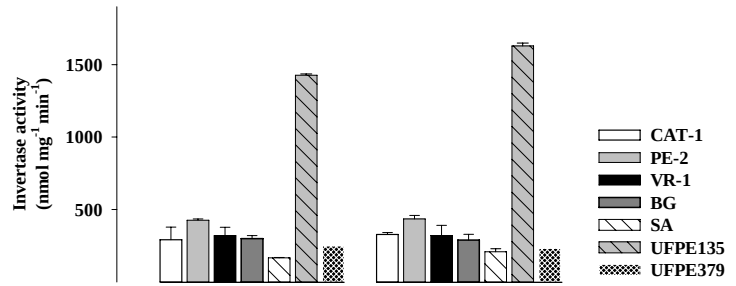
Heat ?

Light ?

pH ?


PE-2
SA-1
VR-1
BG-1
CAT-1
Baker's
Brewer's
Wine
S288C
SNO2
SNO3
SNZ3
SNZ2
MPH3
SOR2
MPH2
SOR1
HXT16
HXT15
SUC2
-5
1:1
+5
Scale


VII
IX


Invertase activity
(nmol mg-1 min-1)
1500
1000
500
CAT-1
PE-2
VR-1
BG
SA
UFPE135
UFPE379

Summary and future developments……..

a-CGH	genomic differences	Fuel yeasts
	selection and/or evolution ?	
Pyridoxine / Thiamin	yeast performance	[sugar] ↑
	vitamin supplementation ?	
	improve YEAST and/or sugar-cane B1 / B6 production ?	

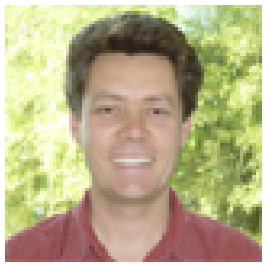
Other industrial yeasts ?

CAPES

CNPq

FAPESP

"Wet" lab



Dr. Gavin Sherlock

"Web" lab

35 Anos Fermentec

Drs. A. Amorim & M.L. Lopes

Dr. J. Finguerut (CTC)

Dr. Carlos A. Rosa (UFMG)

Dr. Marcos A. Morais-Jr (UFPE)