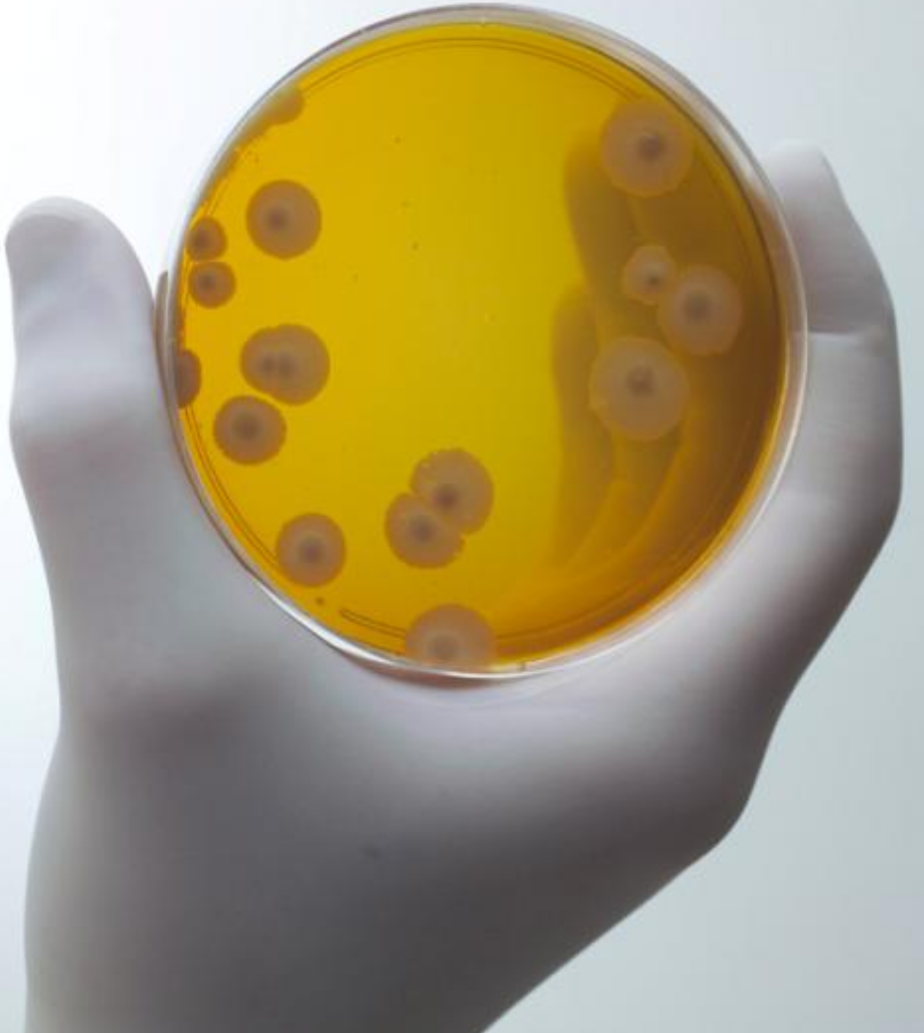
30 YEARS OF FUEL ETHANOL PRODUCTION IN BRAZIL:

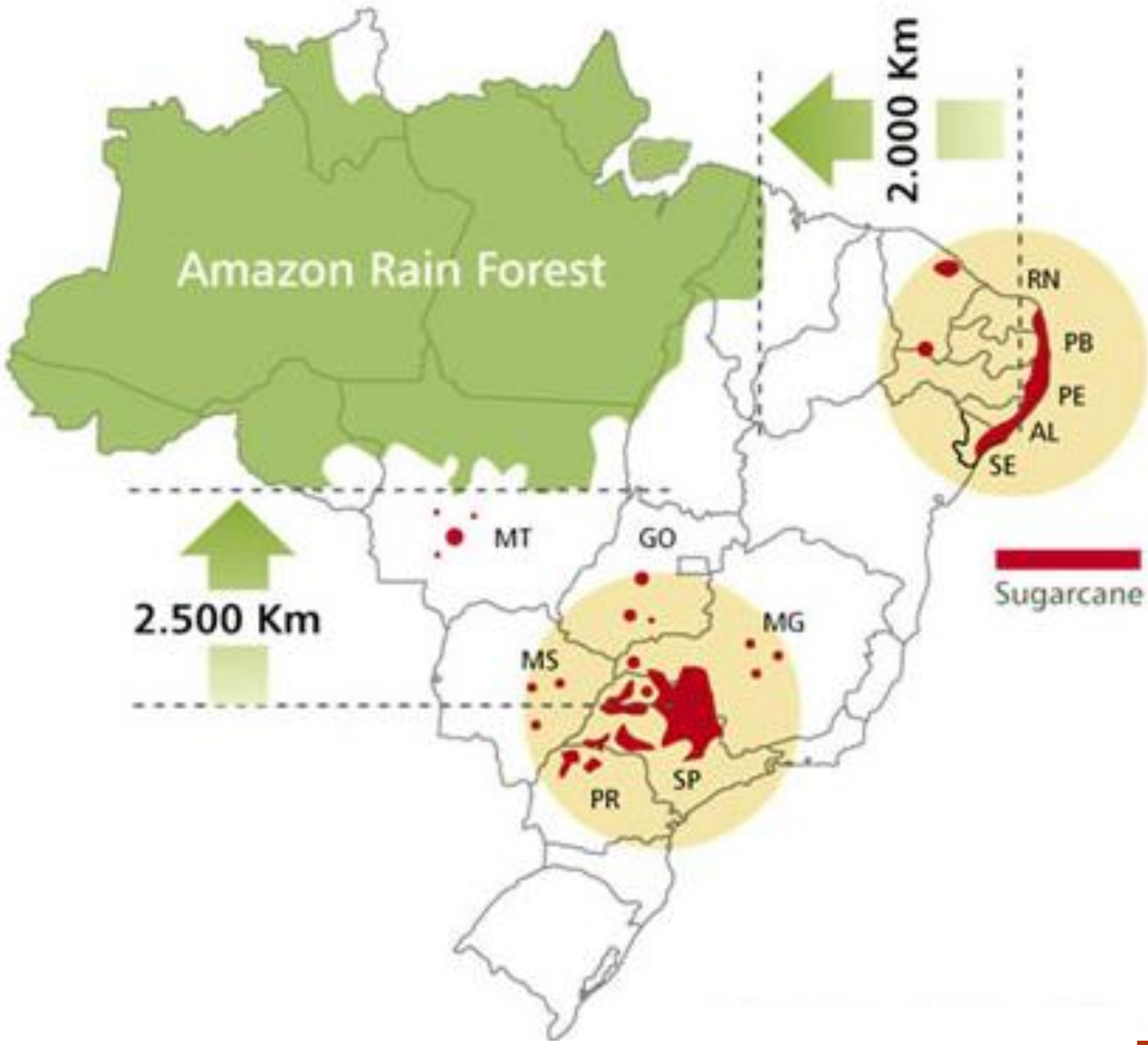
identification and selection of dominant industrial yeast strains



Mário Lúcio Lopes

Fermentec

Sugarcane Production



Source: http://english.unica.com.br/content/show.asp?cntCode={D6C39D36-69BA-458D-A95C-815C87E4404D}

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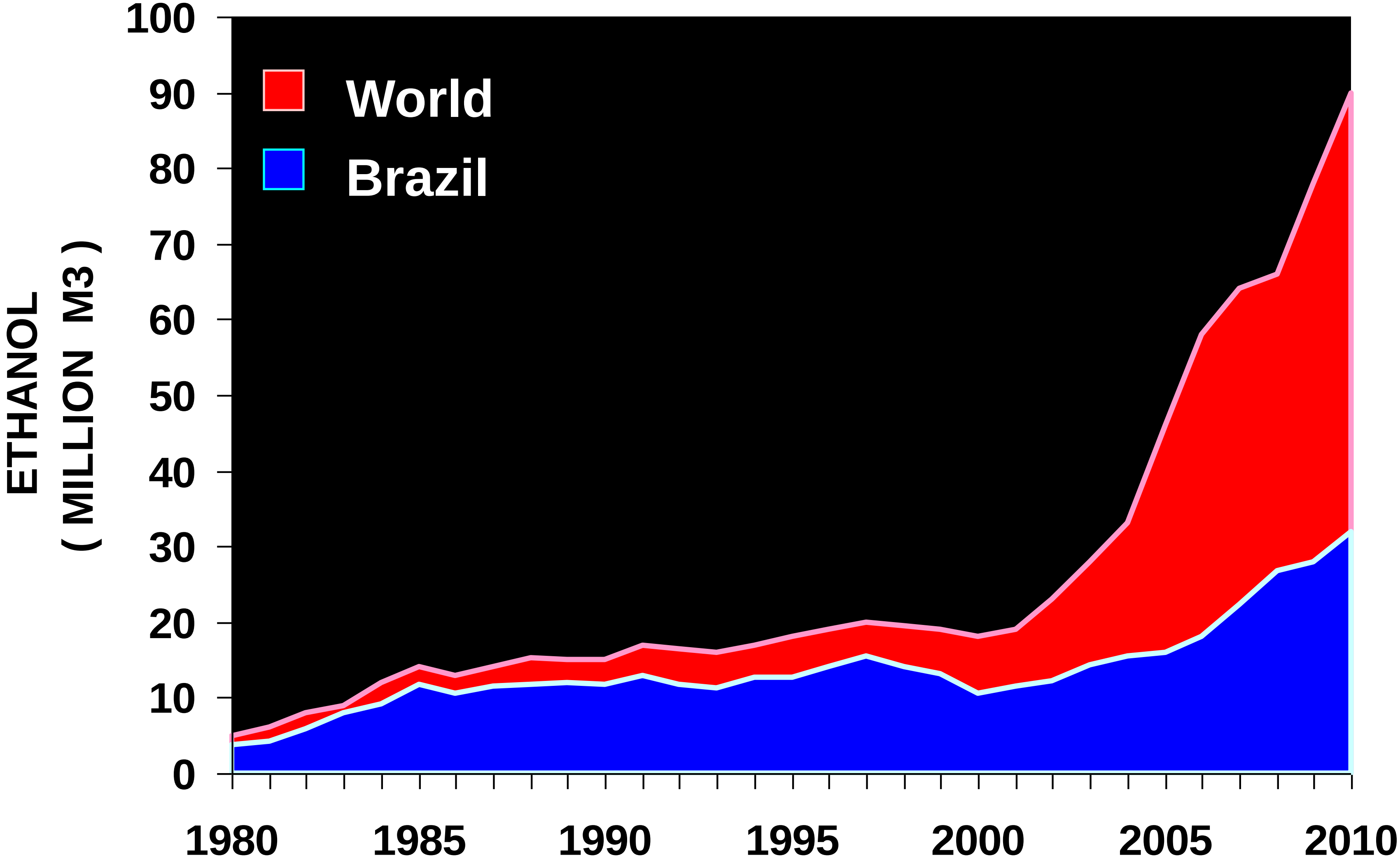
Ethanol Production



Source: Datagro and Bioenergy Review

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Ethanol Production in Brazil

- 420 distilleries: >30 billion liters of ethanol / year
- Selected yeast strains: 154 distilleries
- Fermentor's volume: 0.2 – 3.0 million Liters
- High yeast cell densities: 10-15% (w/v)
- Fermentation time: 6-12 hours
- Yeast cell recycle: 2-3 times/day (250-280 days)
- Ethanol concentration: 8-12% (v/v)
- Fermentation: 85% fed-batch and 15% continuous

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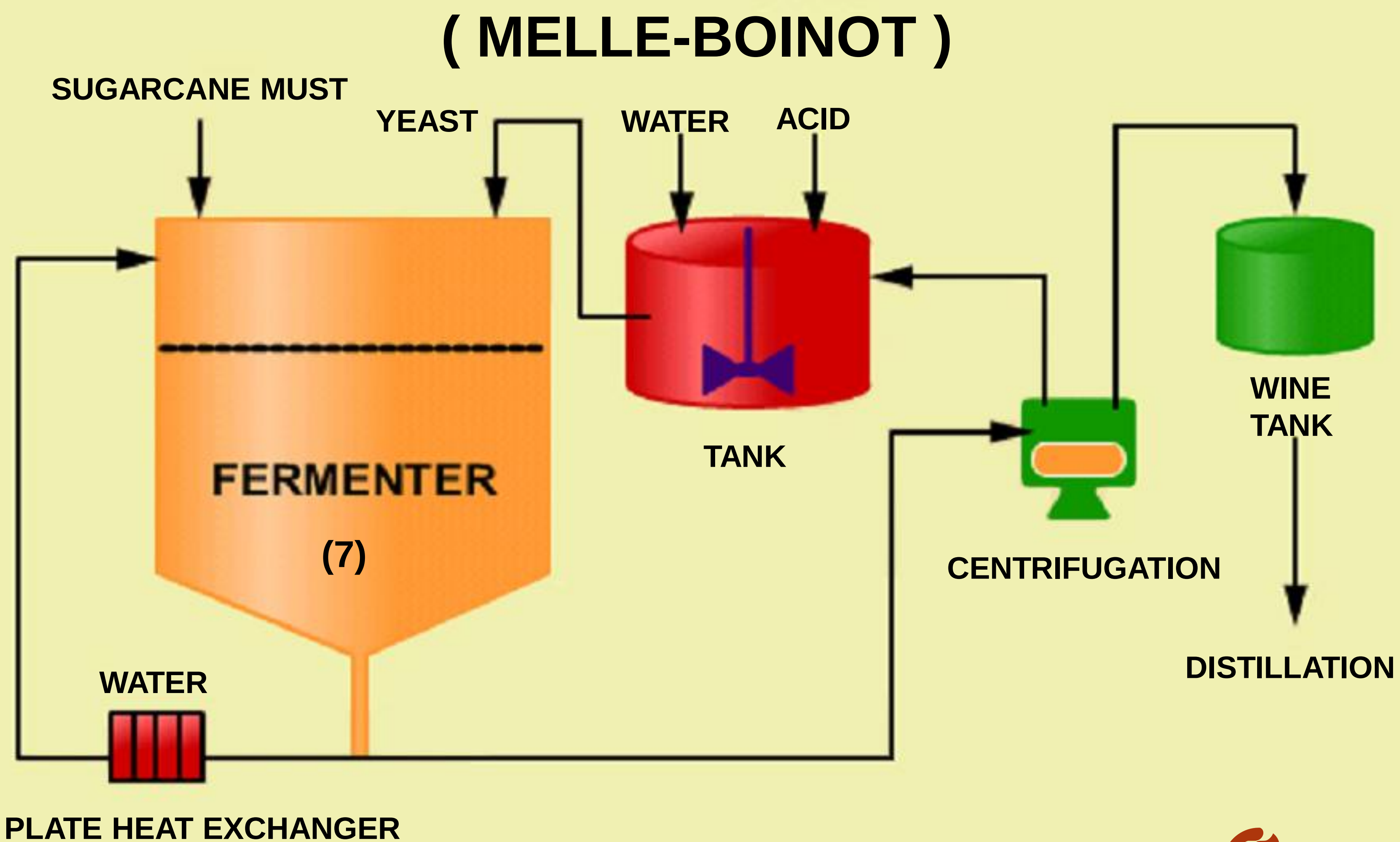
Ethanol Production in Brazil

Fed-batch Fermentation
with yeast recycle by centrifugation



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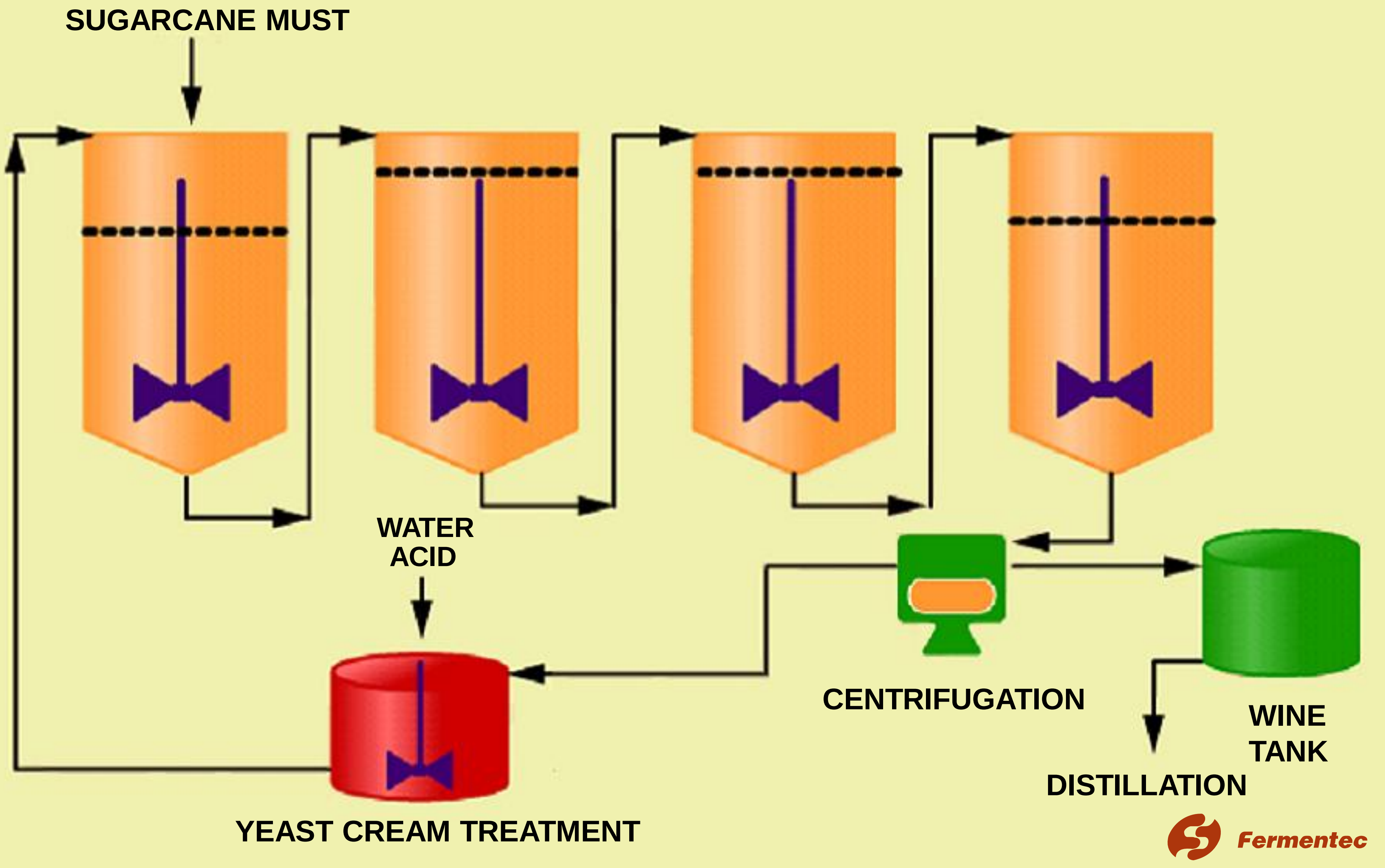
Continuous Fermentation
with yeast recycle by centrifugation



EVOLUTION

OF ETHANOL PRODUCTION

PARAMETERS	1980	2010
Sugarcane production	160 Mln tons	>600 Mln tons
Ethanol production	3.7 Bln liters	>30 Bln liters
Sugar Extraction Yield	88%	96%
Fermentation Yield	75 - 80%	90 - 92%
Distillation Yield	95%	>99%
Bacteria in Wine	10^8-10^9/mL	10^5-10^6/mL
Fermentation Time	18 - 24 h	6 - 12 h

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EVOLUTION
OF ETHANOL PRODUCTION

Several factors contributed to improve the industrial process of ethanol production:

- Sugarcane quality
- Fermentation conditions
- Design of fermentors
- Analytical control of the industrial process
- Reduction of losses
- Control of bacterial contaminants
- **Identification, monitoring and selection of yeast strains**

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1980

YEAST STRAINS
used as starter

- Baker's yeast
- TA-79 (MA-300)
- IZ-1904 (ESALQ-USP)

Until 1989 it was not possible to identify and monitor well the yeast strains among *Saccharomyces*.

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1989

YEAST SELECTION
karyotyping technique

- **Pierre Barre and Françoise Vezinhet**

Institut Supérieur des Products de la Vigne et du Vin

Montpellier, France

- **Luiz Carlos Basso**

Escola Superior de Agricultura Luiz de Queiroz – USP

Piracicaba, SP - Brazil

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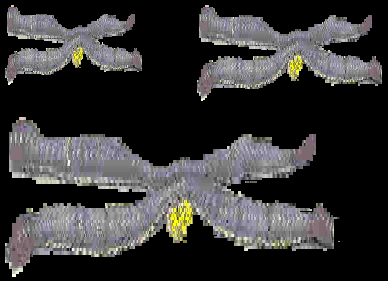
1990

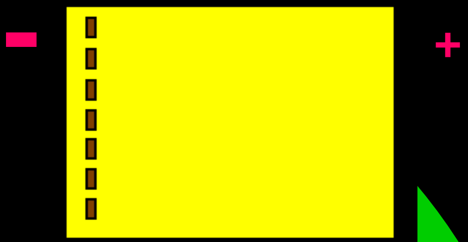
YEAST SELECTION
first analysis

Yeast Colonies

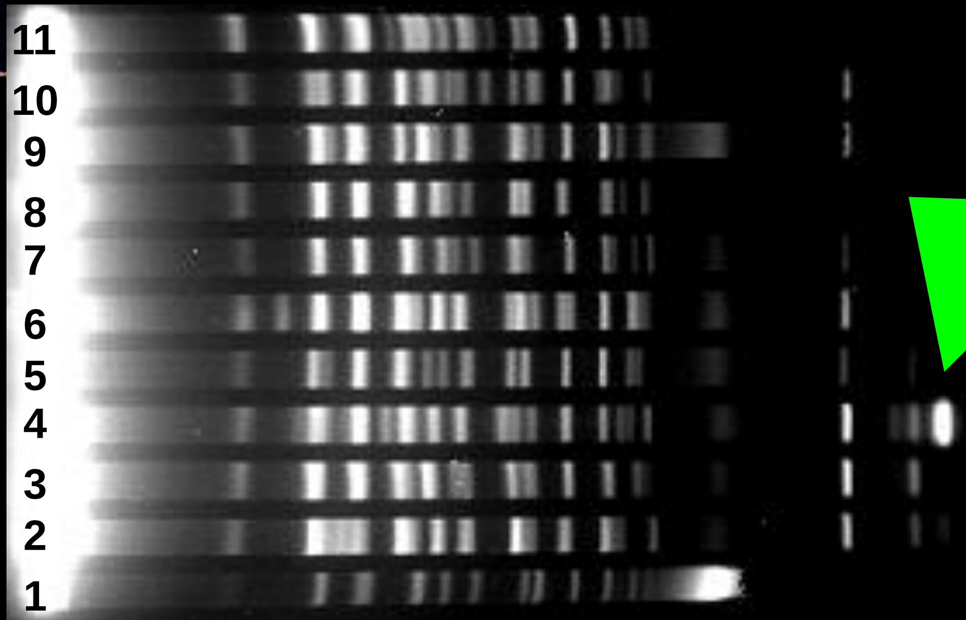
Chromosomes



Electrophoresis



Results



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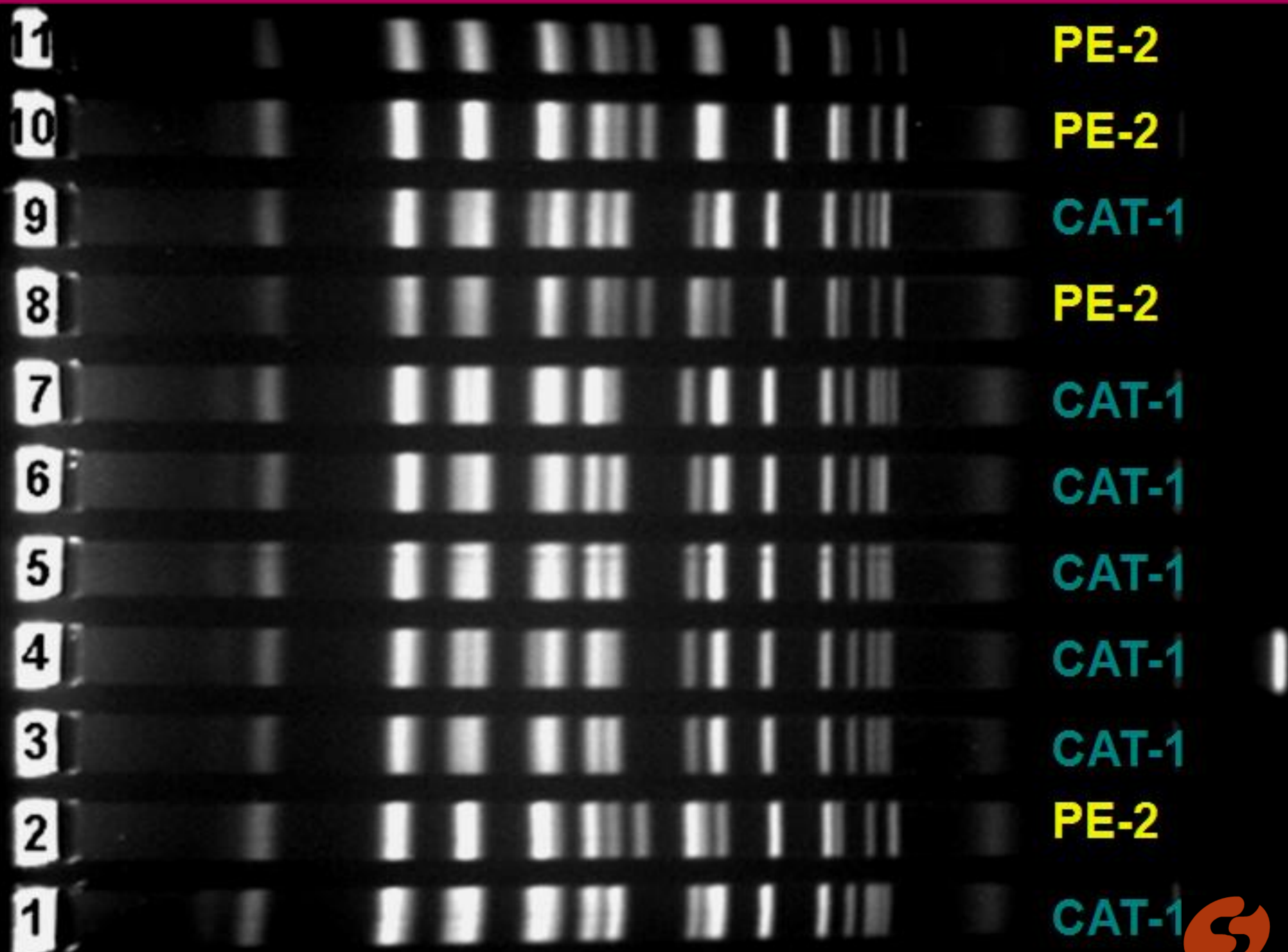
YEAST SELECTION
karyotyping and monitoring



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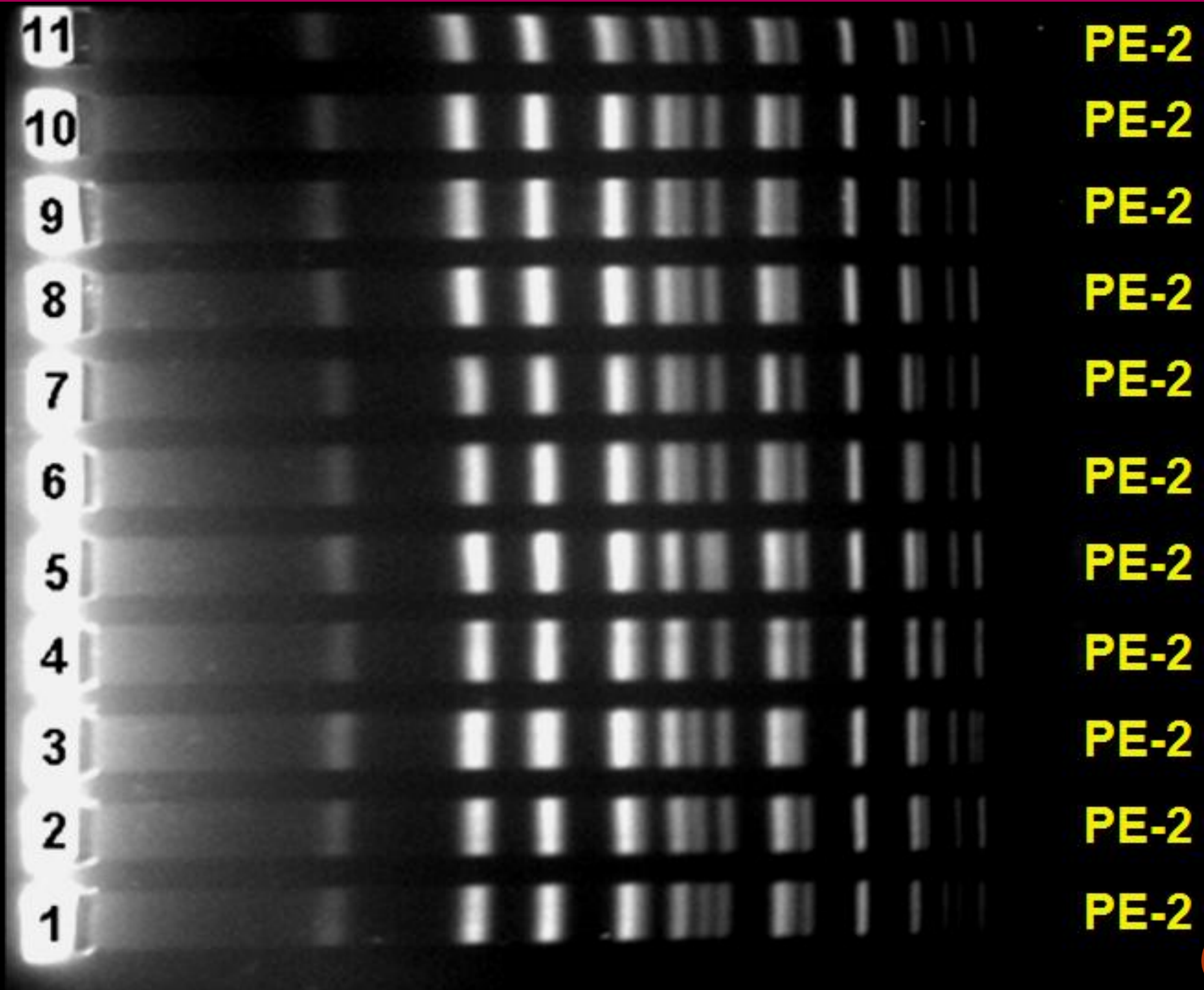
YEAST SELECTION
karyotyping and monitoring



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YEAST SELECTION
first results

- Baker's yeast and laboratory strains were quickly replaced by wild yeast
- Some wild strains of *Saccharomyces* were dominant and persistent in the fermentors
- 62 yeast strains were selected from industrial fermentations and evaluated in laboratory

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YEAST STRAIN EVALUATION
(Laboratory scale)

PARAMETERS	YEAST STRAINS			
	PE-2	VR-1	CAT-1	Baker's Yeast
Fermentation Yield (%)	91.0	90.5	91.2	88.1
Glycerol (%)	3.38	3.20	3.54	4.70
Trehalose (%)	9.5	10.6	10.3	6.0
Viability (%)	94	95	97	61

* AVERAGE OF 6 FERMENTATION CYCLES

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YEAST STRAIN EVALUATION
(Industrial scale - 1995)

STARTER YEASTS (Kg)	
Baker's yeast	2,000
PE-2	0.5
VR-1	0.5
SA-1	0.5

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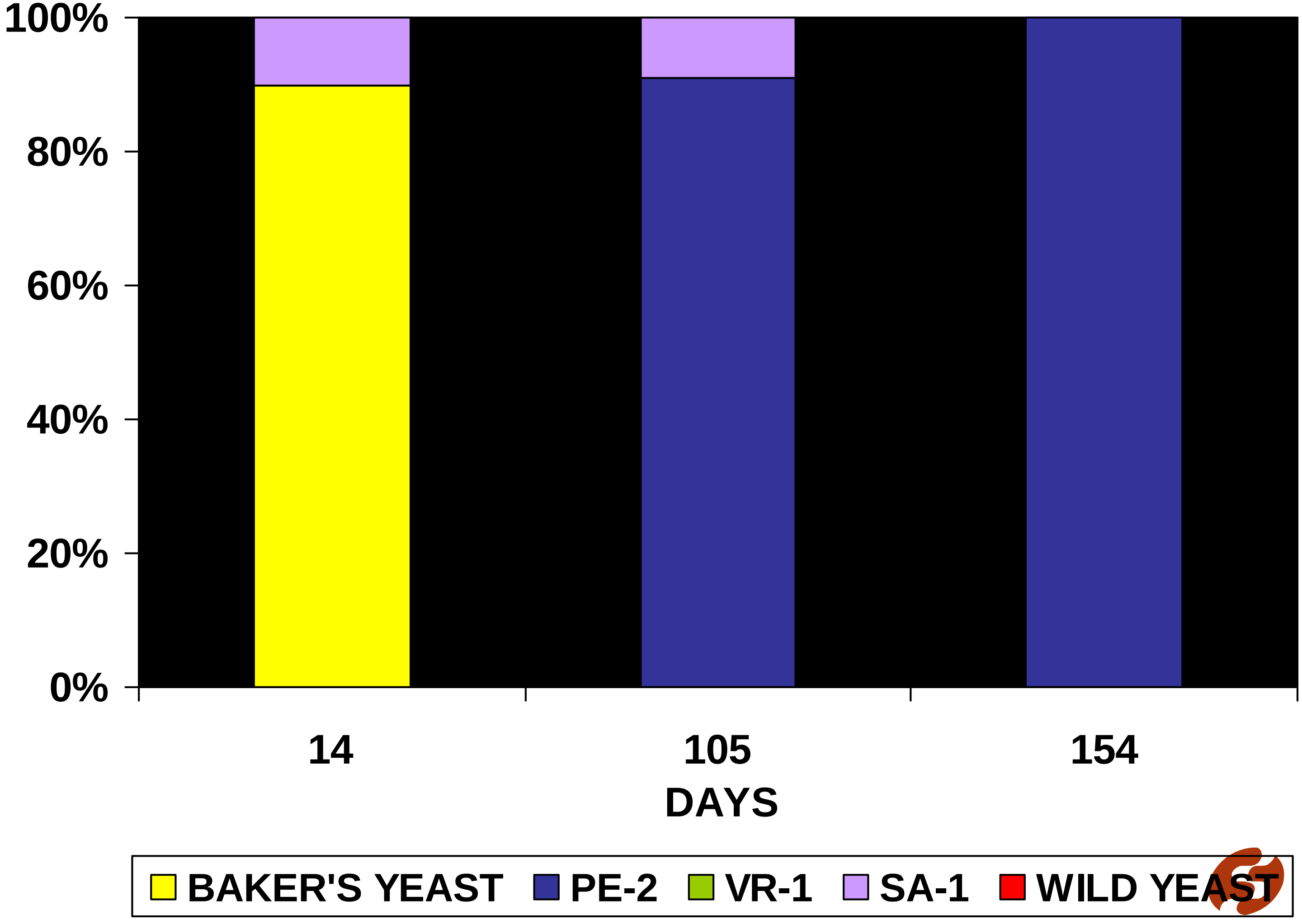
YEAST STRAIN EVALUATION
(Industrial scale - 1995)



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YEAST STRAIN EVALUATION
(Industrial scale - 1995)

STARTER YEASTS (Kg)	
Baker's yeast	10,000
PE-2	0.5
VR-1	0.5
SA-1	0.5

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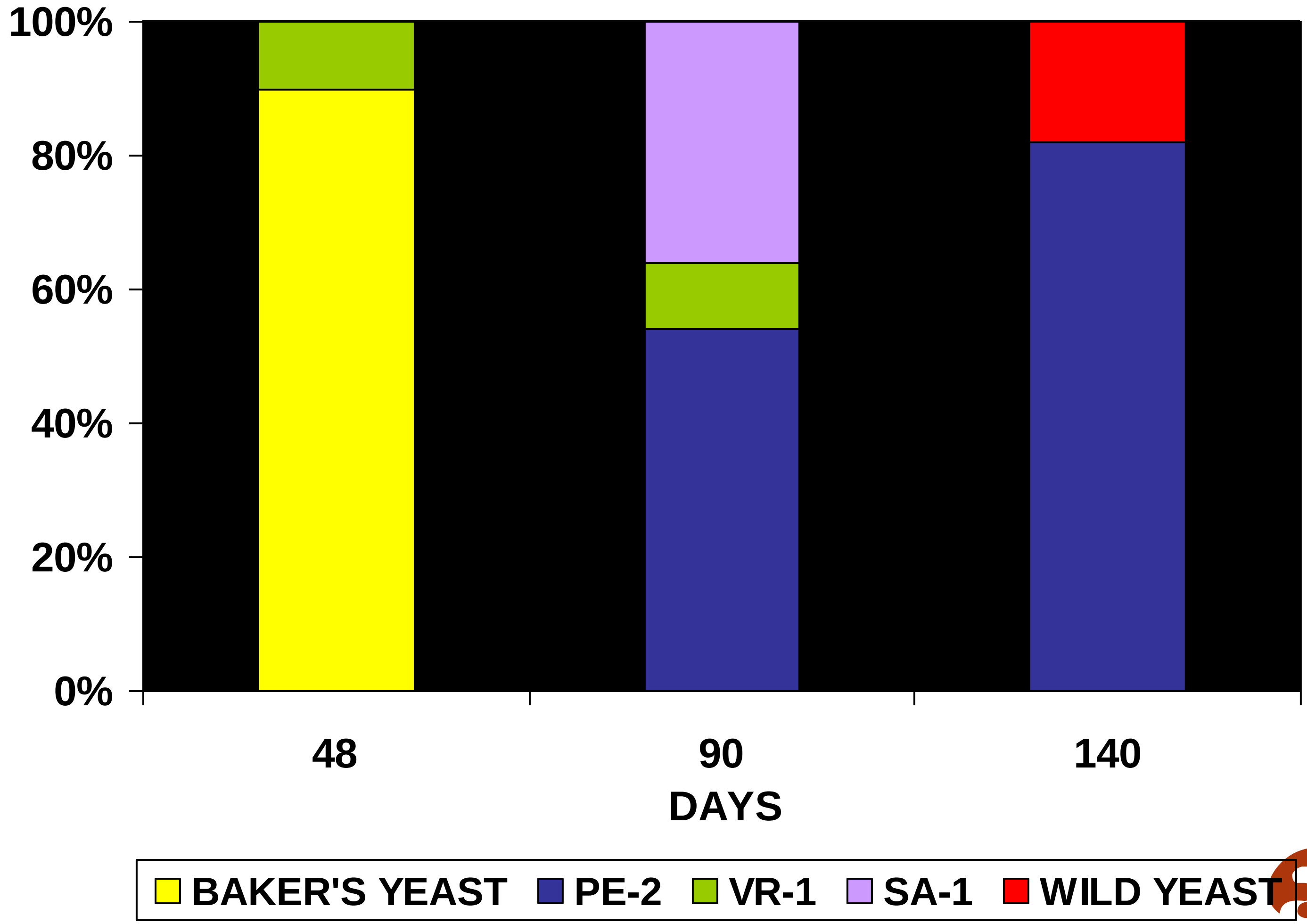
YEAST STRAIN EVALUATION
(Industrial scale - 1995)



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YEAST STRAIN EVALUATION
(Industrial scale - 1997)

STARTER YEASTS (Kg)	
Baker's yeast	10,000
PE-2	100
VR-1	100

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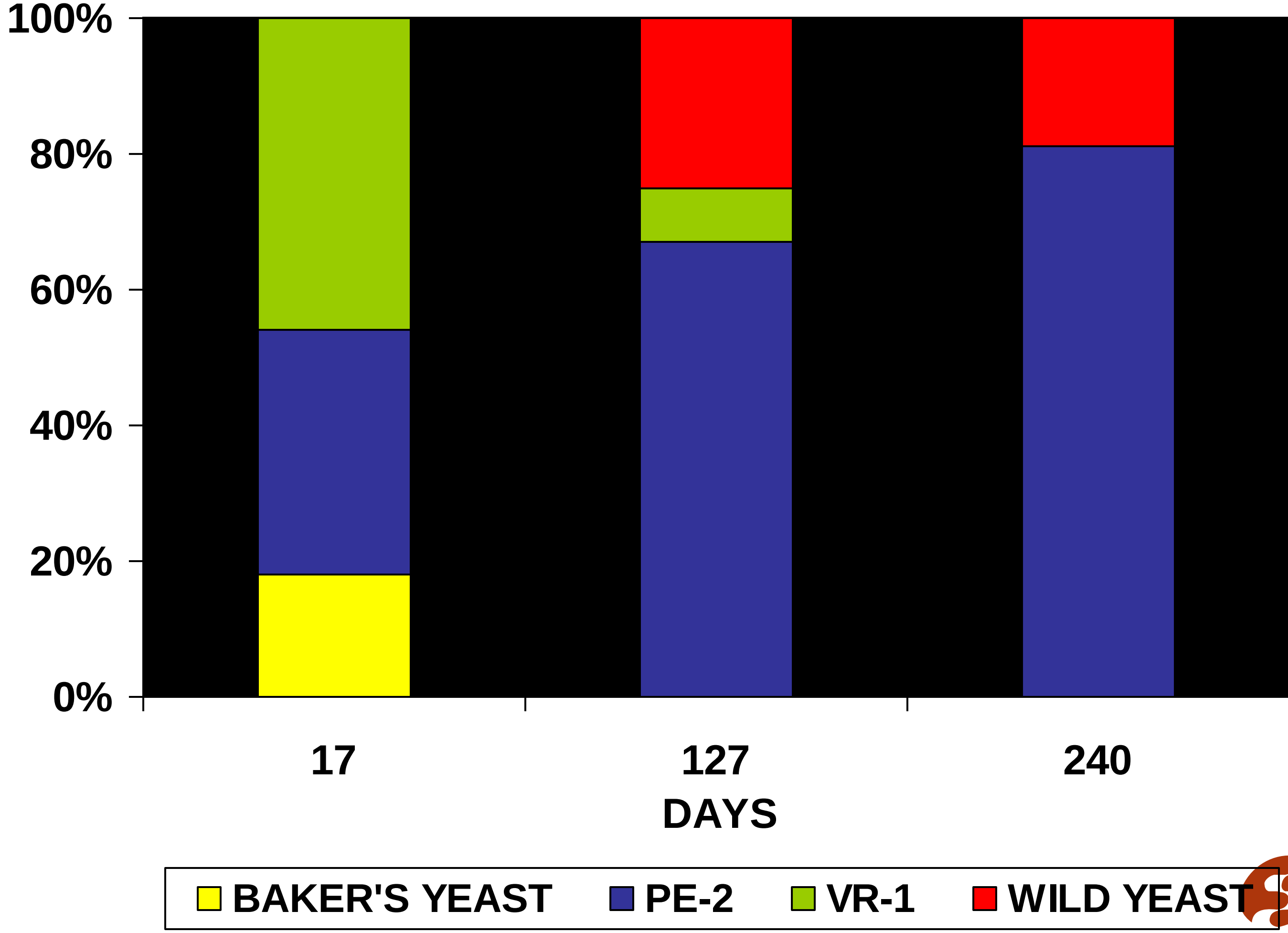
YEAST STRAIN EVALUATION
(Industrial scale - 1997)



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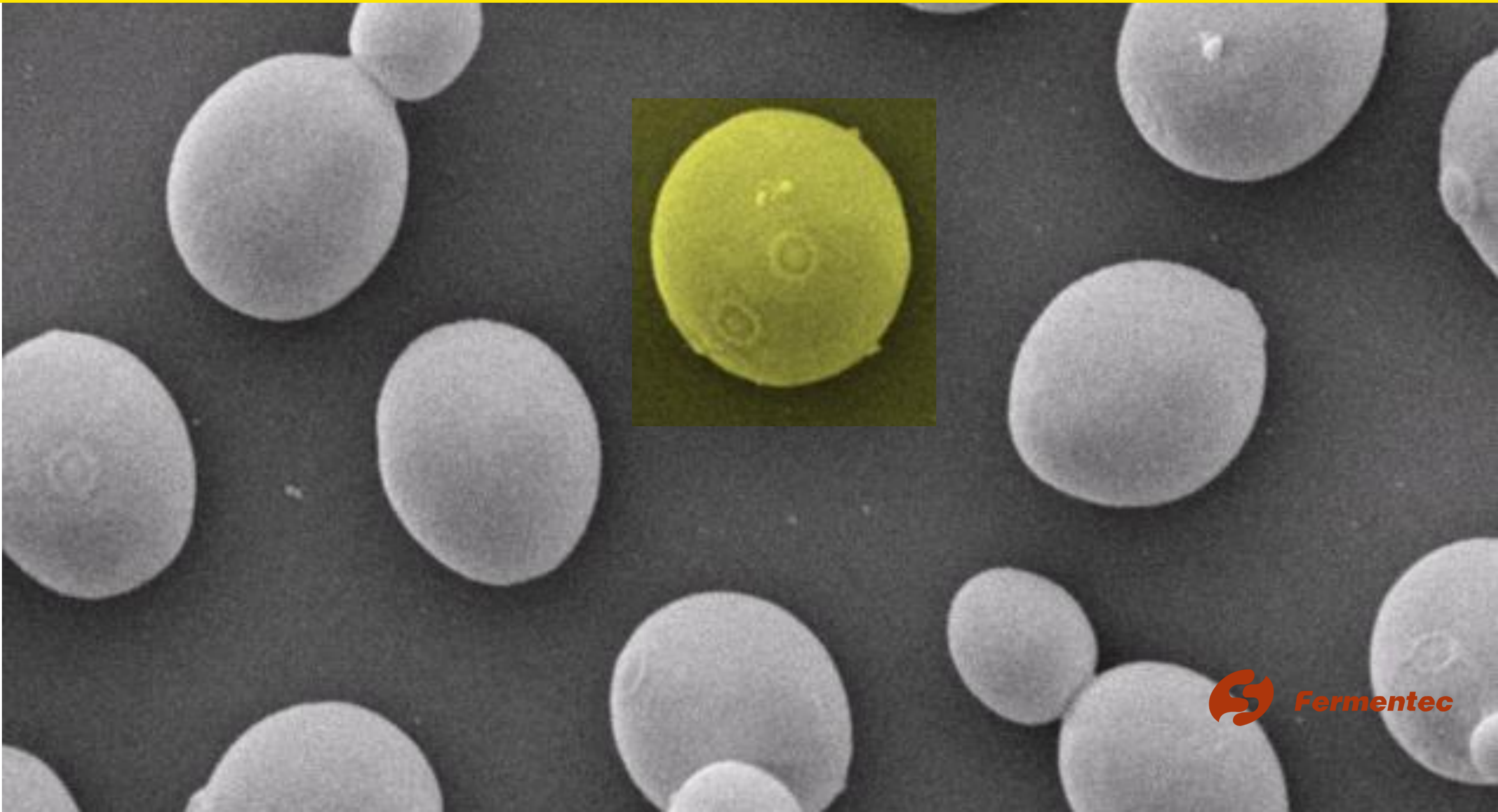
SELECTED X WILD YEAST



ADVANTAGES

OF THE SELECTED YEAST STRAINS

- Tolerance to fermentative recycles
- High fermentation yield
- These strains are not flocculating
- Low foam production

CHARACTERISTICS

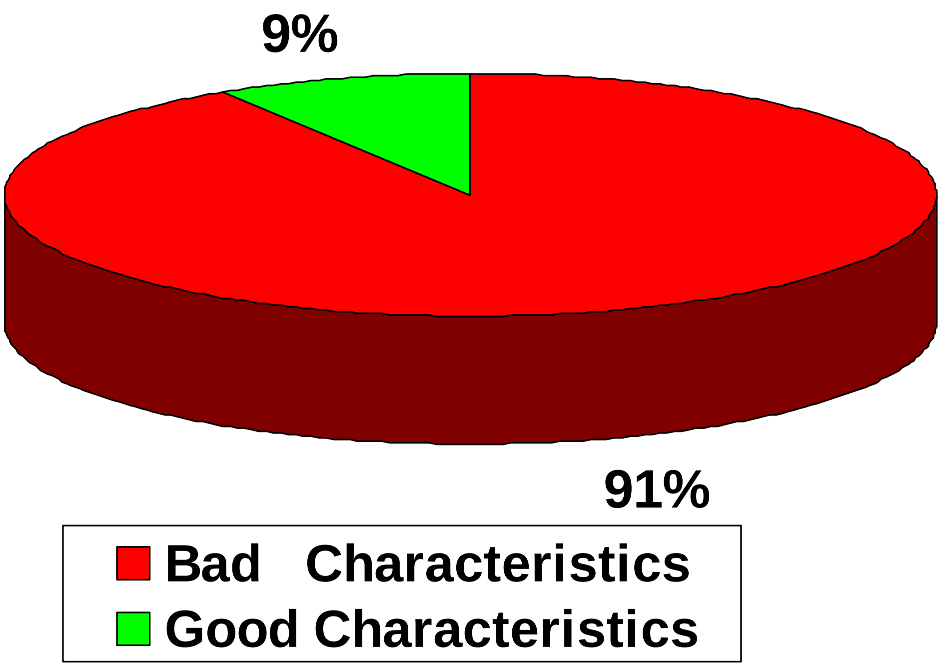
OF 379 WILD YEAST STRAINS

BAD CHARACTERISTICS

Foam: 58%

Flocculation: 37%

Residual sugar: 53%



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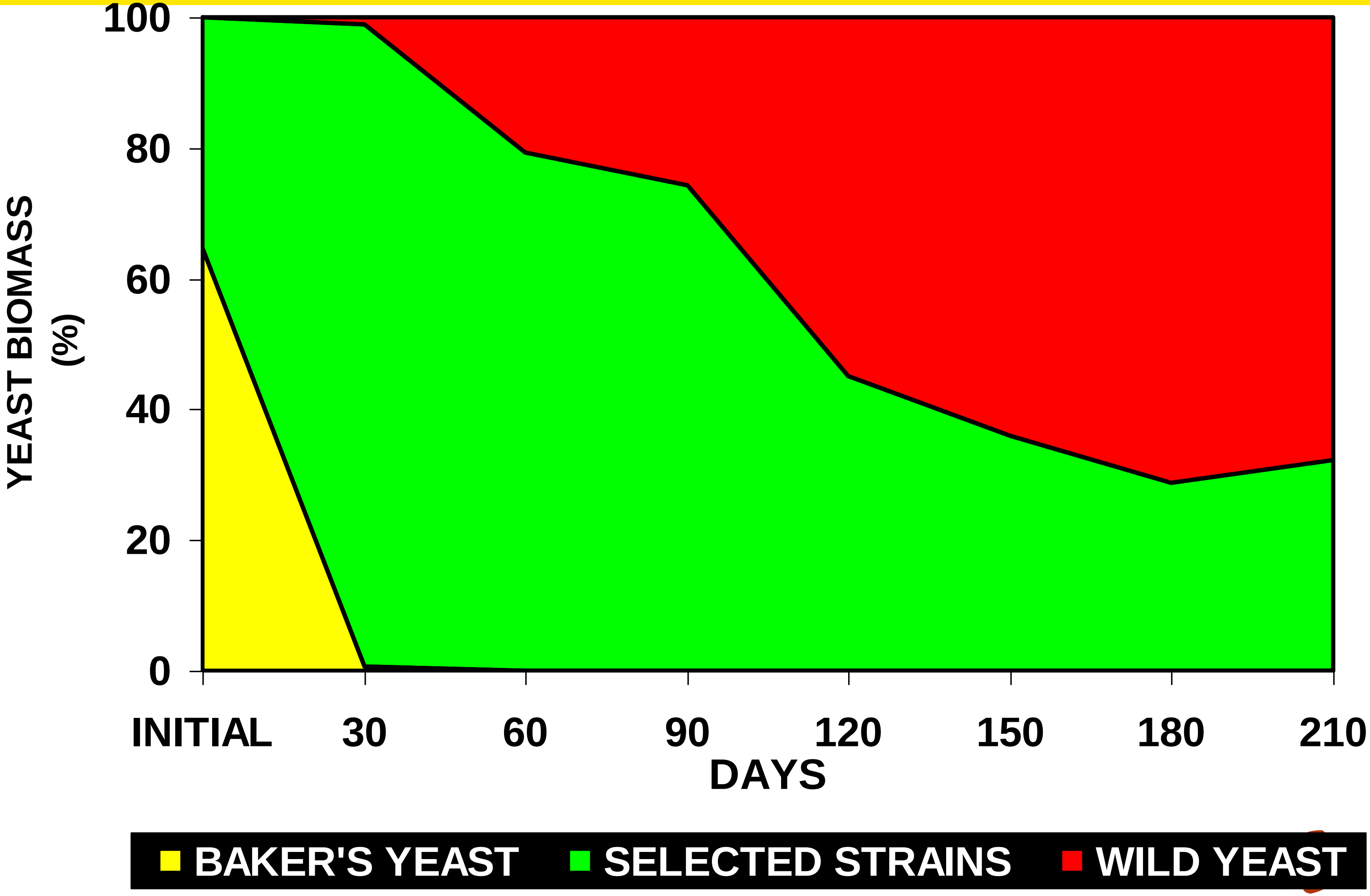
TOLERANCE TO RECYCLES

(Selected strains X Baker's yeast)

78 distilleries - 2003



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TOLERANCE TO RECYCLES
(Selected strains X Baker's yeast)
78 distilleries - 2003

- **Baker's yeast does not survive more than 30 days in industrial processes for ethanol production.**

- **The use of selected yeast strains delay the contamination and competition by wild strains.**

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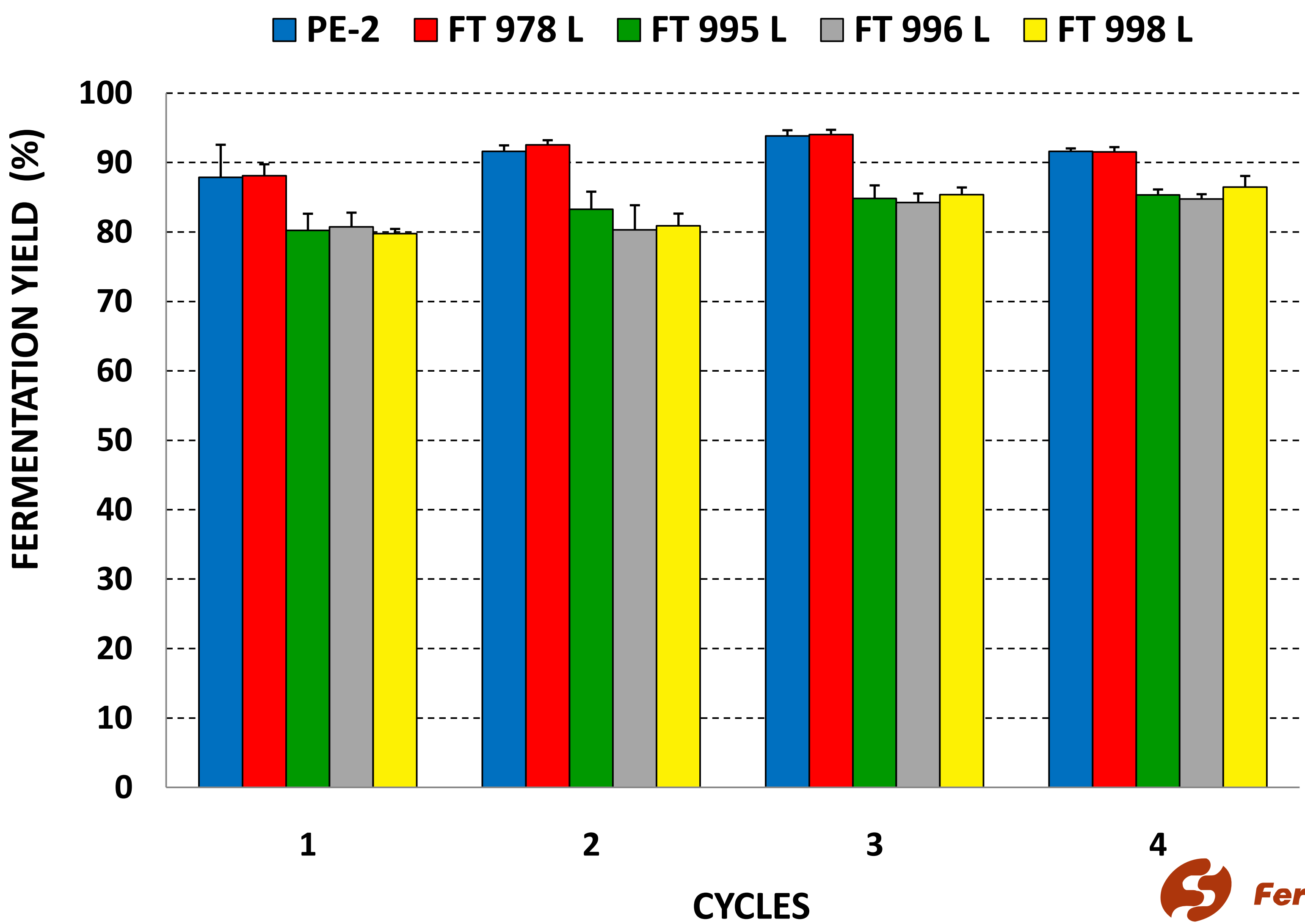
HIGH FERMENTATION YIELD



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HIGH FERMENTATION YIELD

A reduction of 5% in the fermentation yield represents 25,000 L of ethanol per day for a plant that produces 100 million liters/crop season

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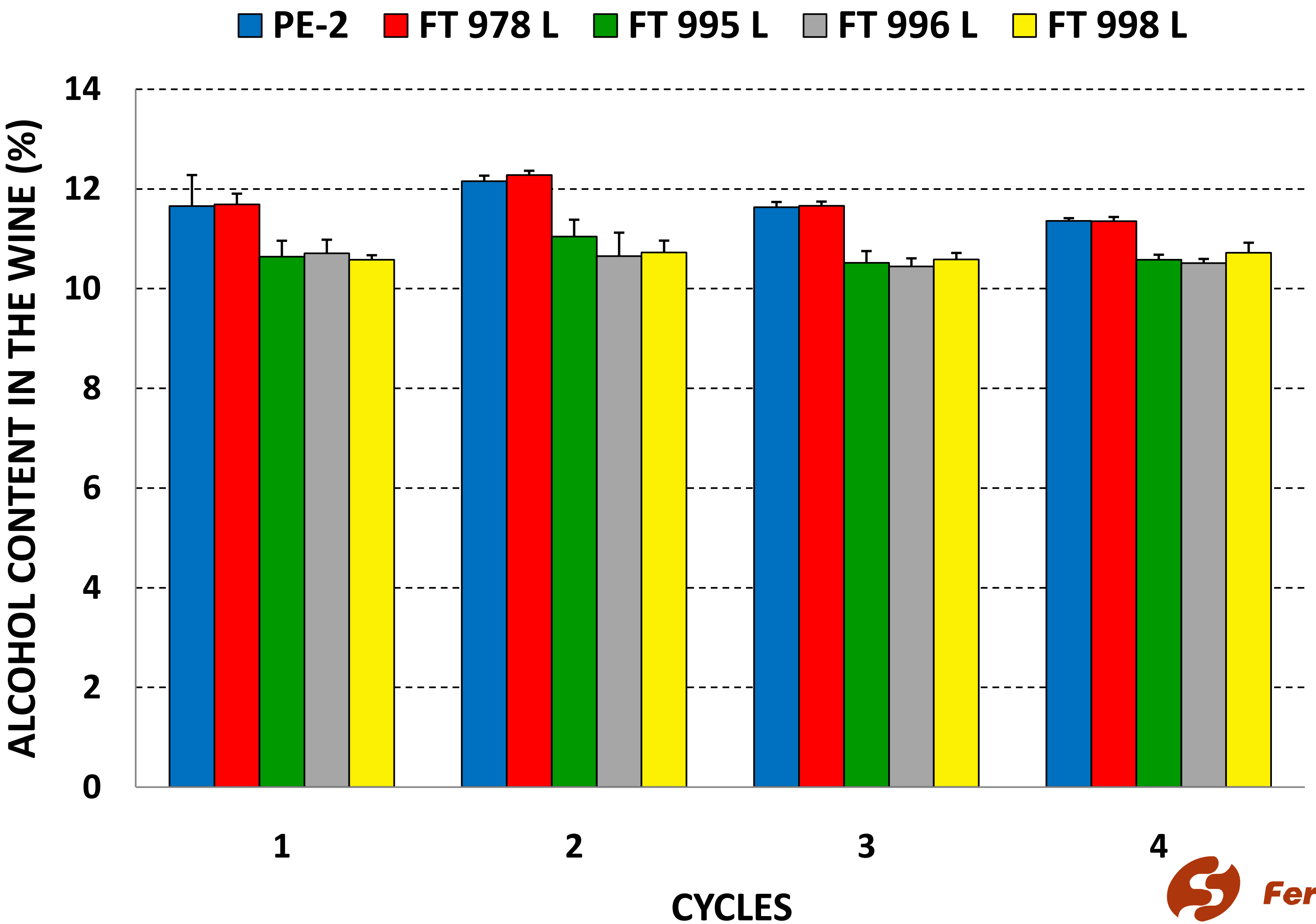
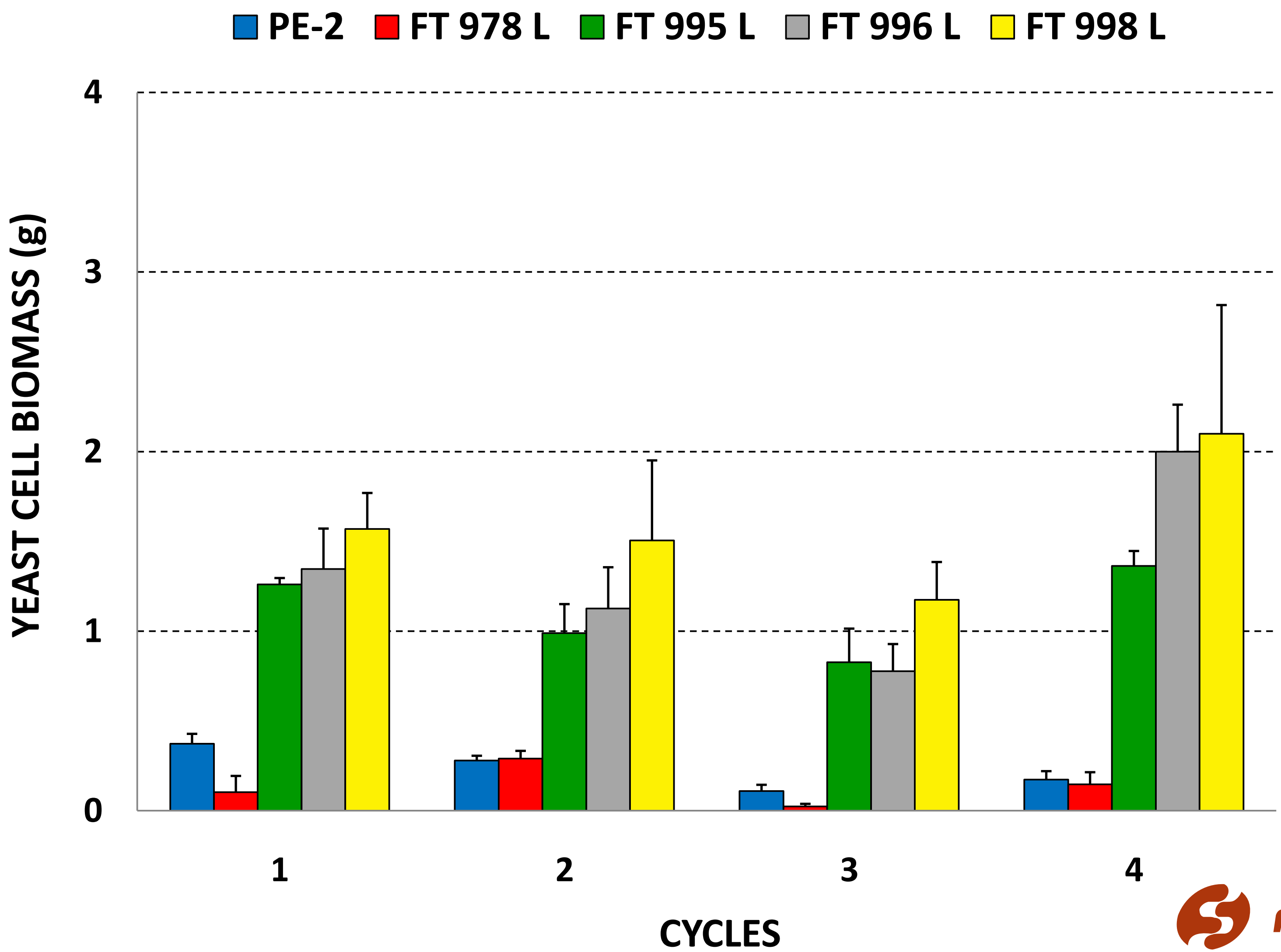
HIGH FERMENTATION YIELD



HIGH FERMENTATION YIELD



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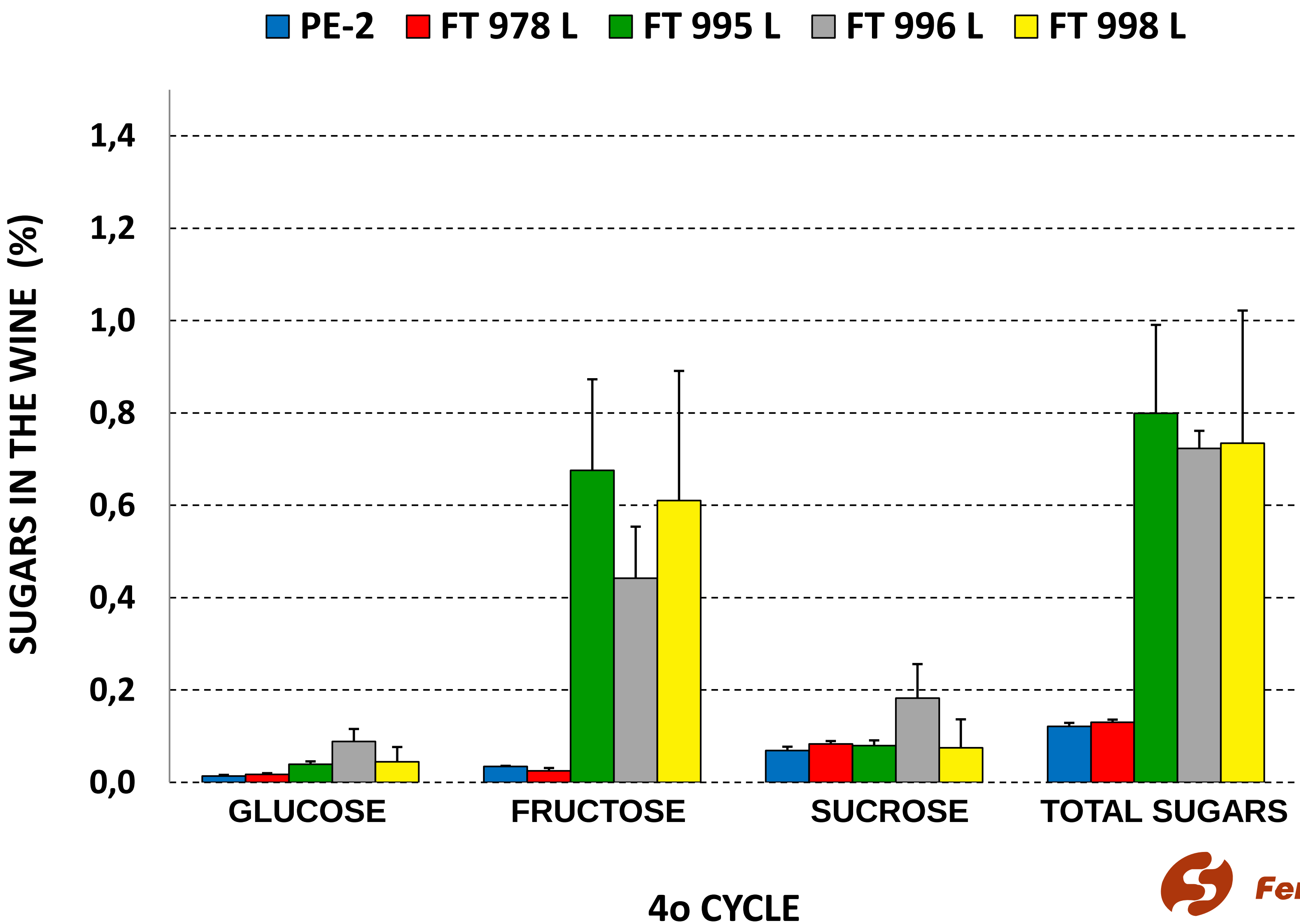
HIGH FERMENTATION YIELD



HIGH FERMENTATION YIELD

0.5% OF NON-FERMENTED SUGARS IN THE WINE REPRESENTS **5 TONS** OF WASTED SUGAR TO EACH **8 HOURS** FOR A TANK OF 1.000 M3

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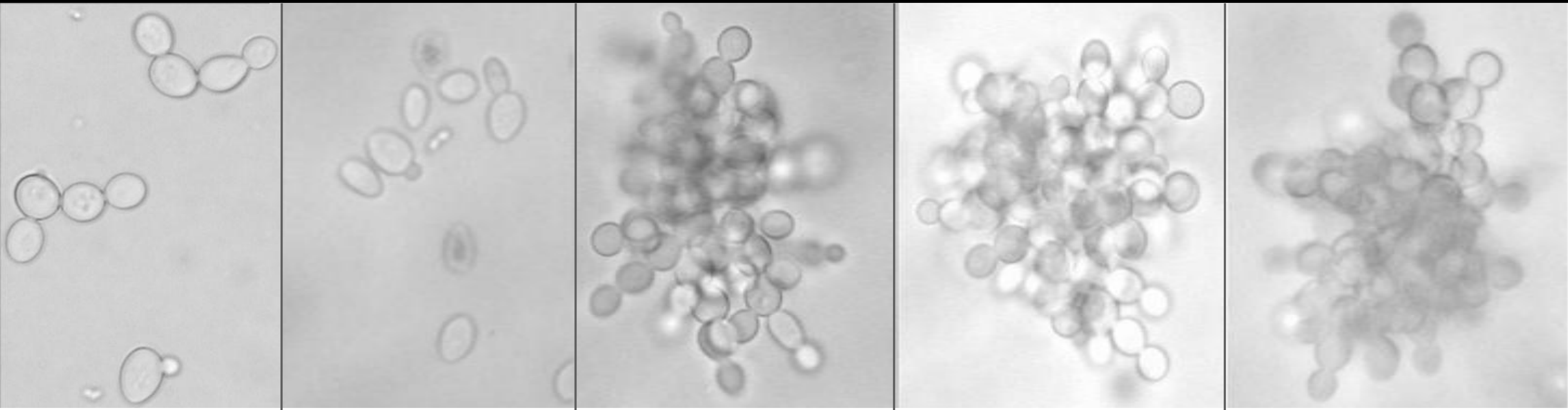
HIGH FERMENTATION YIELD



PE-2 | FT 978 L | FT 995 L | FT 996 L | FT 998 L

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FLOCCULATION

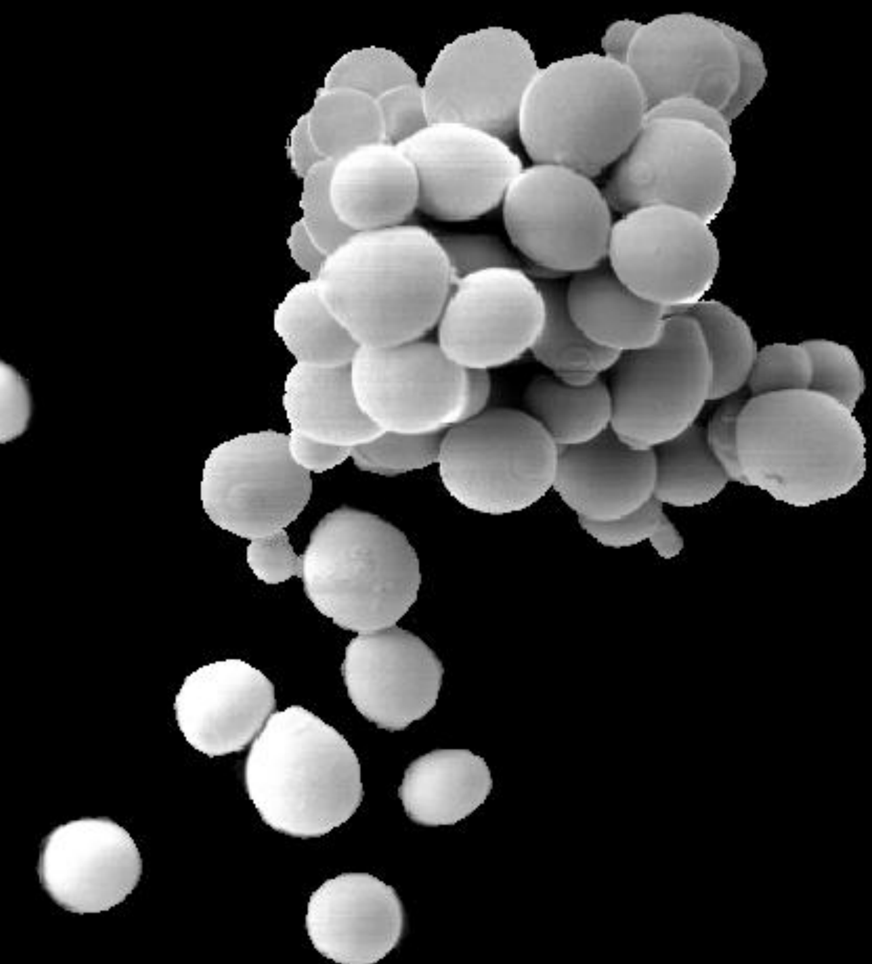
WITHOUT FLOCCULATION

CAT1

INTENSE FLOCCULATION

WILD YEAST

FLOCCULATION



CHAIN FORMATION

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SELECTED YEAST STRAINS PRODUCE LOW FOAM

CAT1

WILD YEAST

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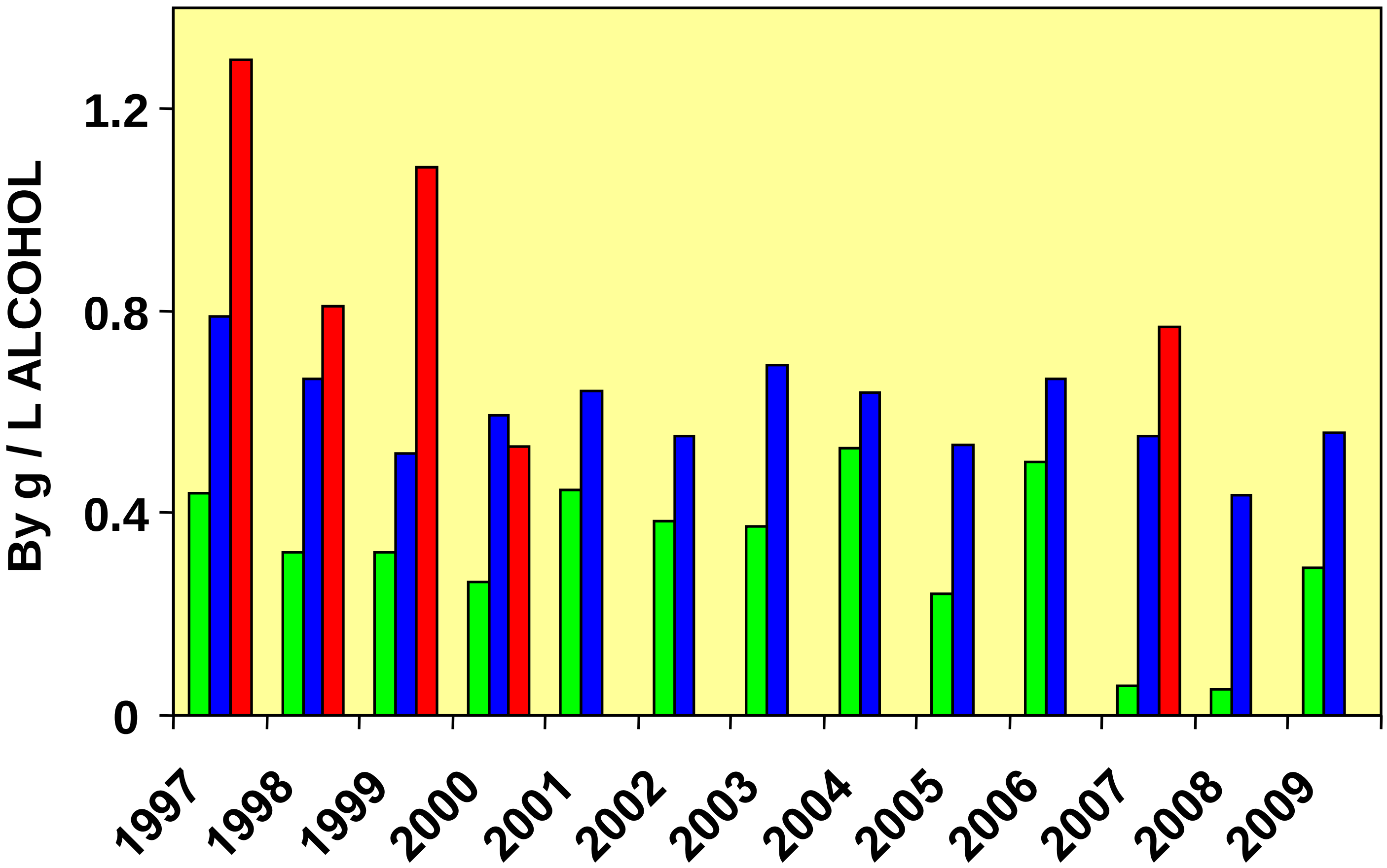
Antifoam Consumption



Distilleries that used selected yeasts

Distilleries where selected yeasts were replaced by wild strains

Distilleries that used Backer's yeasts only

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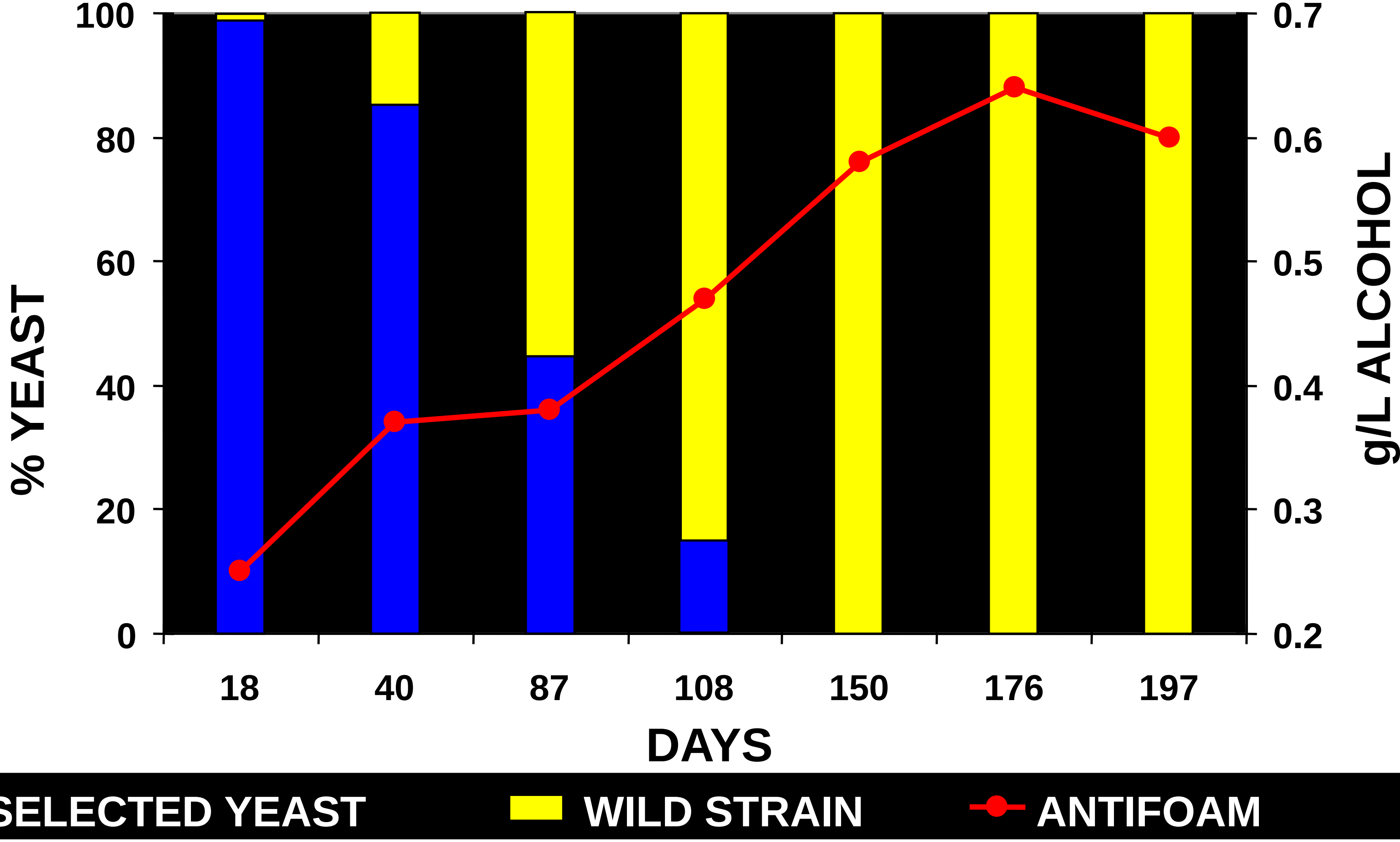
Contamination by other yeasts

ANTIFOAM CONSUMPTION



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ECONOMY WITH ANTIFOAM

In 2009 Fermentec clients saved 1,140 tons of antifoaming by the use of selected yeast strains

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CHARACTERISTICS

	PE2	VR1	CAT1
Ploidy	Diploid	Diploid	Diploid
Sporulation	+	+	+
Heterothallic	+	+	+
Killer	-	-	-
Mitochondrial DNA	+	+	+
Plasmidial DNA	?	?	?

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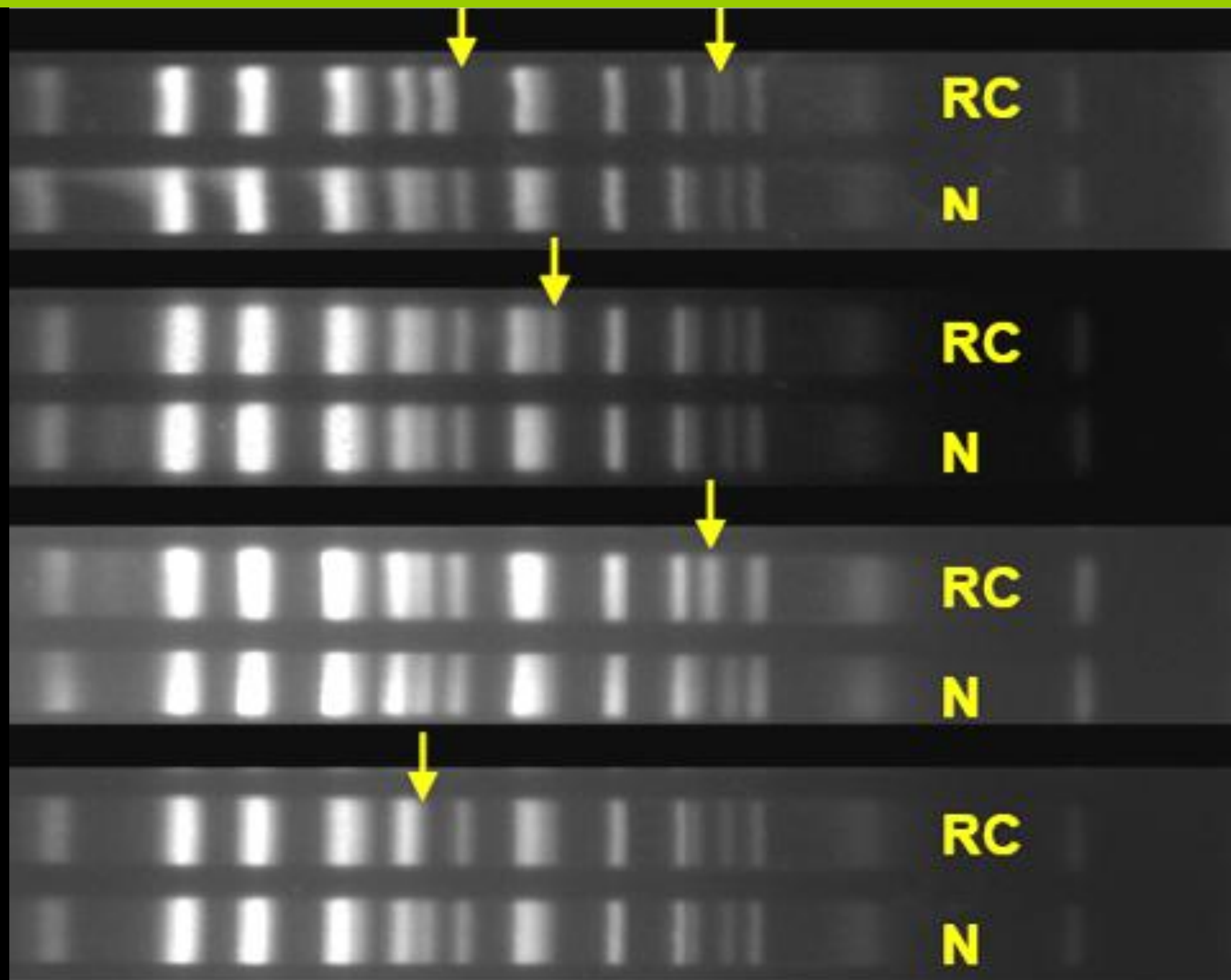
CHROMOSOMAL REARRANGEMENTS

DISTILLERY A

DISTILLERY B

DISTILLERY C

DISTILLERY D



CAT1

2009

Letter

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

Boris U. Stambuk,[1,2,3] Barbara Dunn,[1] Sergio L. Alves Jr.,[2] Eduarda H. Duval,[2] and Gavin Sherlock[1,3]

[1]*Department of Genetics, Stanford University, Stanford, California 94305-5120, USA;* [2]*Departamento de Bioquímica, Universidade Federal de Santa Catarina, Florianópolis, Santa Catarina 88040-900, Brazil*

Fuel ethanol is now a global energy commodity that is competitive with gasoline. Using microarray-based comparative genome hybridization (aCGH), we have determined gene copy number variations (CNVs) common to five industrially important fuel ethanol *Saccharomyces cerevisiae* strains responsible for the production of billions of gallons of fuel ethanol per year from sugarcane. These strains have significant amplifications of the telomeric *SNO* and *SNZ* genes, which are involved in the biosynthesis of vitamins B6 (pyridoxine) and B1 (thiamin). We show that increased copy number of these genes confers the ability to grow more efficiently under the repressing effects of thiamin, especially in medium lacking pyridoxine and with high sugar concentrations. These genetic changes have likely been adaptive and selected for in the industrial environment, and may be required for the efficient utilization of biomass-derived sugars from other renewable feedstocks.

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CAT1

2010

Diploid genome sequence of the industrial fuel ethanol fermentative *Saccharomyces cerevisiae* strain CAT-1

Chunlin Wang[1*], Farbod Babrzadeh[1*], Roxana Jalili1, Shadi Shokralla[1], Sarah Pierce[1], Avi Robinson-Mosher[1], Pål Nyren[2], Robert W. Shafer[3], Luiz C. Basso[4], Henrique V. Amorim[5], Antonio J. de Oliveira[5], Ronald W. Davis[1], Boris U. Stambuk[6,7], Mostafa Ronaghi[1] and Baback Gharizadeh[1*]

[1]Stanford Genome Technology Center, Stanford University, USA
[2]Department of Biochemistry at School of Biotechnology, KTH Royal Institute of Technology, Sweden
[3]Department of Medicine, School of Medicine, Stanford University, USA
[4]Biological Science Department, Escola Superior de Agricultura Luiz de Queiroz, USP, Piracicaba, SP, Brazil
[5]Fermentec Ltda., Piracicaba, SP, Brazil
[6]Department of Genetics, School of Medicine, Stanford University, CA 94305-5120, USA
[7]Departmento de Bioquímica, Centro de Ciências Biológicas, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil

BMC Genomics
http://www.biomedcentral.com

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PE2

2009

Letter

Genome structure of a *Saccharomyces cerevisiae* strain widely used in bioethanol production

Juan Lucas Argueso,[1,9,10] Marcelo F. Carazzolle,[3,9] Piotr A. Mieczkowski,[6,9] Fabiana M. Duarte,[3] Osmar V.C. Netto,[3] Silvia K. Missawa,[3] Felipe Galzerani,[3] Gustavo G.L. Costa,[3] Ramon O. Vidal,[3] Melline F. Noronha,[3] Margaret Dominska,[1] Maria G.S. Andrietta,[4] Sílvio R. Andrietta,[4] Anderson F. Cunha,[5] Luiz H. Gomes,[7] Flavio C.A. Tavares,[7] André R. Alcarde,[8] Fred S. Dietrich,[1,2] John H. McCusker,[1] Thomas D. Petes,[1] and Gonçalo A.G. Pereira[3,10]

[1]Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, North Carolina 27710, USA; [2]Institute for Genome Sciences and Policy, Duke University Medical Center, Durham, North Carolina 27710, USA; [3]Laboratório de Genômica e Expressão, Departamento de Genética e Evolução, Instituto de Biologia, Universidade Estadual de Campinas, Campinas-São Paulo 13083-970, Brazil; [4]Laboratório de Biotecnologia e Bioprocessos, Centro Pluridisciplinar de Pesquisas Químicas e Biológicas, Universidade Estadual de Campinas, Campinas-São Paulo 13081-970, Brazil; [5]Departamento de Genética e Evolução, CCBS, Universidade Federal de São Carlos, São Carlos-São Paulo 13565-905, Brazil; [6]Department of Genetics, School of Medicine, University of North Carolina, Chapel Hill, North Carolina 27599, USA; [7]Departmento de Genética, Universidade de São Paulo, Piracicaba-São Paulo 13418-900, Brazil; [8]Departamento de Agroindústria, Alimentos e Nutrição, Escola Superior de Agricultura "Luiz de Queiroz," Universidade de São Paulo, Piracicaba-São Paulo 13418-900, Brazil

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2ª GENERATION YEAST

MAIN CHARACTERISTICS

- **High fermentative yield**
- **Tolerant to high ethanol concentration in the wine**
- **Tolerant to low pH**
- **Non-flocculating / low foam**
- **Faster fermentations**
- **High viability during recycles**
- **Derived from PE2**

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SELECTION OF NEW YEAST STRAINS

- To obtain strains more adapted to industrial fermentations.
- To extend the number of strains available to distilleries
- To search new strains for different characteristics and industrial purposes.
- Preservation of these strains in a specialized Culture Collection (Fermentec)

Fermentec

CULTURE COLLECTION

- 4,000 yeast strains identified by karyotyping and mitochondrial DNA per year
- 2,000 yeast and bacteria from industrial processes of ethanol production
- Conservation:
  - Lyophilized cells in glass ampuls
  - Ultra-low temperatute (freezer and liquid N)
  - Liquid medium

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THANK YOU VERY MUCH

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