30 YEARS OF FUEL ETHANOL PRODUCTION IN BRAZIL: identification and selection of dominant industrial yeast strains

Mário Lúcio Lopes

Fermentec
Sugarcane Production

Ethanol Production

Source: Datagro and Bioenergy Review
## 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
Ethanol Production in Brazil
Fed-batch Fermentation with yeast recycle by centrifugation

( MELLE-BOINOT )

SUGARCANE MUST → YEAST → WATER → ACID → FERMENTER (7) → TANK → CENTRIFUGATION → DISTILLATION → WINE TANK

PLATE HEAT EXCHANGER
Continuous Fermentation with yeast recycle by centrifugation
## EVOLUTION OF ETHANOL PRODUCTION

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>1980</th>
<th>2010</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugarcane production</td>
<td>160 Mln tons</td>
<td>&gt;600 Mln tons</td>
</tr>
<tr>
<td>Ethanol production</td>
<td>3.7 Bln liters</td>
<td>&gt;30 Bln liters</td>
</tr>
<tr>
<td>Sugar Extraction Yield</td>
<td>88%</td>
<td>96%</td>
</tr>
<tr>
<td>Fermentation Yield</td>
<td>75 - 80%</td>
<td>90 - 92%</td>
</tr>
<tr>
<td>Distillation Yield</td>
<td>95%</td>
<td>&gt;99%</td>
</tr>
<tr>
<td>Bacteria in Wine</td>
<td>$10^8$-$10^9$/mL</td>
<td>$10^5$-$10^6$/mL</td>
</tr>
<tr>
<td>Fermentation Time</td>
<td>18 - 24 h</td>
<td>6 - 12 h</td>
</tr>
</tbody>
</table>
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
Until 1989 it was not possible to identify and monitor well the yeast strains among *Saccharomyces*.

- Baker’s yeast
- TA-79 (MA-300)
- IZ-1904 (ESALQ-USP)
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
YEAST SELECTION

first analysis

1990

Yeast Colonies

Chromosomes

Electrophoresis

Results
YEAST SELECTION

karyotyping and monitoring
YEAST SELECTION
karyotyping and monitoring
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.
YEAST STRAIN EVALUATION
(Laboratory scale)

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>YEAST STRAINS</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PE-2</td>
<td>VR-1</td>
<td>CAT-1</td>
<td>Baker’s Yeast</td>
</tr>
<tr>
<td>Fermentation Yield (%)</td>
<td>91.0</td>
<td>90.5</td>
<td>91.2</td>
<td>88.1</td>
</tr>
<tr>
<td>Glycerol (%)</td>
<td>3.38</td>
<td>3.20</td>
<td>3.54</td>
<td>4.70</td>
</tr>
<tr>
<td>Trehalose (%)</td>
<td>9.5</td>
<td>10.6</td>
<td>10.3</td>
<td>6.0</td>
</tr>
<tr>
<td>Viability (%)</td>
<td>94</td>
<td>95</td>
<td>97</td>
<td>61</td>
</tr>
</tbody>
</table>

* AVERAGE OF 6 FERMENTATION CYCLES
YEAST STRAIN EVALUATION
(Industrial scale - 1995)

<table>
<thead>
<tr>
<th>STARTER YEASTS (Kg)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Baker’s yeast</td>
<td>2,000</td>
</tr>
<tr>
<td>PE-2</td>
<td>0.5</td>
</tr>
<tr>
<td>VR-1</td>
<td>0.5</td>
</tr>
<tr>
<td>SA-1</td>
<td>0.5</td>
</tr>
</tbody>
</table>
YEAST STRAIN EVALUATION
(Industrial scale - 1995)
### YEAST STRAIN EVALUATION
(Industrial scale - 1995)

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</tr>
</thead>
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<td>Baker’s yeast</td>
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</tr>
<tr>
<td>PE-2</td>
<td>0.5</td>
</tr>
<tr>
<td>VR-1</td>
<td>0.5</td>
</tr>
<tr>
<td>SA-1</td>
<td>0.5</td>
</tr>
</tbody>
</table>
YEAST STRAIN EVALUATION
(Industrial scale - 1995)

Graph showing the evaluation of different yeast strains over 140 days. The graph compares Baker's Yeast, PE-2, VR-1, SA-1, and Wild Yeast. The data indicates the percentage of each strain over time.
### YEAST STRAIN EVALUATION
(Industrial scale - 1997)

<table>
<thead>
<tr>
<th>STARTER YEASTS (Kg)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Baker’s yeast</td>
<td>10,000</td>
</tr>
<tr>
<td>PE-2</td>
<td>100</td>
</tr>
<tr>
<td>VR-1</td>
<td>100</td>
</tr>
</tbody>
</table>
YEAST STRAIN EVALUATION
(Industrial scale - 1997)

- BAKER'S YEAST
- PE-2
- VR-1
- WILD YEAST

DAYS
17
127
240
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%

Diagram: 91% Good Characteristics, 9% Bad Characteristics

Legend:
- Red: Bad Characteristics
- Green: Good Characteristics
TOLERANCE TO RECYCLES
(Selected strains X Baker’s yeast)
78 distilleries - 2003

YEAST BIOMASS (%)

INITIAL 30 60 90 120 150 180 210
DAYS

YEAST BIOMASS (%)

INITIAL 30 60 90 120 150 180 210
DAYS

BAKER'S YEAST SELECTED STRAINS WILD YEAST

Fermentec
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.
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.
HIGH FERMENTATION YIELD

ALCOHOL CONTENT IN THE WINE (%)

<table>
<thead>
<tr>
<th>CYCLES</th>
<th>PE-2</th>
<th>FT 978 L</th>
<th>FT 995 L</th>
<th>FT 996 L</th>
<th>FT 998 L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
HIGH FERMENTATION YIELD

![Graph showing yeast cell biomass over cycles with different fermentation systems.](image-url)

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

**Yeast Cell Biomass (g)**

- Cycles 1 to 4 with corresponding biomass values for each system.
HIGH FERMENTATION YIELD

SUGARS IN THE WINE (%)

GLUCOSE      FRUCTOSE      SUCROSE      TOTAL SUGARS

4o CYCLE
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
FLOCCULATION

WITHOUT FLOCCULATION

INTENSE FLOCCULATION

CAT1

WILD YEAST
SELECTED YEAST STRAINS PRODUCE LOW FOAM

CAT1

WILD YEAST
Antifoam Consumption

- Distilleries that used selected yeasts
- Distilleries where selected yeasts were replaced by wild strains
- Distilleries that used Backer’s yeasts only
Contamination by other yeasts

ANTIFOAM CONSUMPTION

% YEAST

SELECTED YEAST

WILD STRAIN

ANTIFOAM

DAYS

G/L ALCOHOL

0 0.2 0.4 0.6 0.8 1

18 40 87 108 150 176 197
In 2009 Fermentec clients saved 1,140 tons of antifoaming by the use of selected yeast strains.
## CHARACTERISTICS

<table>
<thead>
<tr>
<th></th>
<th>PE2</th>
<th>VR1</th>
<th>CAT1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ploidy</strong></td>
<td>Diploid</td>
<td>Diploid</td>
<td>Diploid</td>
</tr>
<tr>
<td><strong>Sporulation</strong></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Heterothallic</strong></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Killer</strong></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Mitochondrial DNA</strong></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Plasmidial DNA</strong></td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
</tbody>
</table>
CHROMOSOMAL REARRANGEMENTS

DISTILLERY A

DISTILLERY B

DISTILLERY C

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

Chunlin Wang¹*, Farbod Babrzadeh¹*, Roxana Jalili¹, Shadi Shokralla¹, Sarah Pierce¹, Avi Robinson-Mosher¹, Pål Nyren², Robert W. Shafer³, Luiz C. Basso⁴, Henrique V. Amorim⁵, Antonio J. de Oliveira⁵, Ronald W. Davis¹, Boris U. Stambuk⁶,⁷, Mostafa Ronaghi¹ and Baback Gharizadeh¹*

1Stanford Genome Technology Center, Stanford University, USA
2Department of Biochemistry at School of Biotechnology, KTH Royal Institute of Technology, Sweden
3Department of Medicine, School of Medicine, Stanford University, USA
4Biological Science Department, Escola Superior de Agricultura Luiz de Queiroz, USP, Piracicaba, SP, Brazil
5Fermentec Ltda., Piracicaba, SP, Brazil
6Department of Genetics, School of Medicine, Stanford University, CA 94305-5120, USA
7Departamento de Bioquímica, Centro de Ciências Biológicas, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil
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. Pereira³,¹⁰

¹Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, North Carolina 27710, USA; ²Institute for Genome Sciences and Policy, Duke University Medical Center, Durham, North Carolina 27710, USA; ³Laboratório de Genómica e Expressão, Departamento de Genética e Evolução, Instituto de Biologia, Universidade Estadual de Campinas, Campinas-São Paulo 13083-970, Brazil; ⁴Laboratório de Biotecnologia e Bioprocessos, Centro Pluridisciplinar de Pesquisas Químicas e Biológicas, Universidade Estadual de Campinas, Campinas-São Paulo 13081-970, Brazil; ⁵Departamento de Genética e Evolução, CCBS, Universidade Federal de São Carlos, São Carlos-São Paulo 13565-905, Brazil; ⁶Department of Genetics, School of Medicine, University of North Carolina, Chapel Hill, North Carolina 27599, USA; ⁷Department of Genética, Universidade de São Paulo, Piracicaba-São Paulo 13418-900, Brazil; ⁸Departamento de Agroindústria, Alimentos e Nutrição, Escola Superior de Agricultura “Luiz de Queiroz,” Universidade de São Paulo, Piracicaba-São Paulo 13418-900, Brazil
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
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)
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 temperature (freezer and liquid N)
  – Liquid medium
THANK YOU VERY MUCH