Glycerol metabolism and transport activity regulation in *Saccharomyces cerevisiae*

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**gut 1 Δ**

**gut 2 Δ**

**Glucose grown cells**

**CCCP 50 μM**

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**gut 1 Δ**

**Ethanol grown cells**

- CCCP 50 μM

**gut 2 Δ**
Ethanol grown cells

CCCp 50 μM

**gut 1 Δ**

100% Efflux

[Glíc] in / [Glíc] out

Incubation period (min)

**gut 2 Δ**

80 - 90% Efflux

[Glíc] in / [Glíc] out

Incubation period (min)

**wt**

No Efflux

Incubation period (min)

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# Glycerol transport kinetic parameters

## Glucose grown cells

<table>
<thead>
<tr>
<th>Growth phase</th>
<th>Exponential</th>
<th>Pre-stationary</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Strain</strong></td>
<td><strong>Km (mM)</strong></td>
<td><strong>Vmax (μmol h⁻¹ g dwt⁻¹)</strong></td>
</tr>
<tr>
<td>wt IGC 3507</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>gut1 Δ</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>gut2 Δ</td>
<td>Nd</td>
<td>Nd</td>
</tr>
</tbody>
</table>

*In red* - Lages and Lucas 1997

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Absence of saturation kinetics

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### Kinetic parameters from glycerol transport

Mutants from glycerol metabolic pathway

Ethanol grown cells

<table>
<thead>
<tr>
<th>Strain</th>
<th>Km  (mM)</th>
<th>Vmax (μmol.h⁻¹.g dwt⁻¹)</th>
<th>Kd  (μmol.h⁻¹.g dwt⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt 3507</td>
<td>1.1 ± 0.3 (5)</td>
<td>310±92 (5)</td>
<td>N.d.</td>
</tr>
<tr>
<td>wt W303</td>
<td>2.0±0.4 (3)</td>
<td>253±46 (3)</td>
<td>0.006 (2)</td>
</tr>
<tr>
<td>gut1Δ</td>
<td>1.0±0.3 (3)</td>
<td>165±25 (3)</td>
<td>0.019±0.001 (3)</td>
</tr>
<tr>
<td>gut2Δ</td>
<td>1.5±0.7 (3)</td>
<td>212±48 (3)</td>
<td>0.008±0.002 (3)</td>
</tr>
<tr>
<td>gpp1Δ</td>
<td>1.6±0.7 (3)</td>
<td>326±62 (3)</td>
<td>0.011±0.002 (3)</td>
</tr>
<tr>
<td>gpp2Δ</td>
<td>1.9±0.2 (2)</td>
<td>318±20 (2)</td>
<td>0.009±0.001 (2)</td>
</tr>
<tr>
<td>gpp1Δgpp2Δ</td>
<td>1.3±0.3 (3)</td>
<td>226±27 (3)</td>
<td>0.009±0.001 (3)</td>
</tr>
<tr>
<td>gpd1Δ</td>
<td>1.3±0.3 (2)</td>
<td>256±27 (2)</td>
<td>0.008±0.001 (2)</td>
</tr>
<tr>
<td>gpd2Δ</td>
<td>1.2 (1)</td>
<td>252 (1)</td>
<td>0.009 (1)</td>
</tr>
<tr>
<td>gpd1Δgpd2Δ</td>
<td>1.6±0.4 (3)</td>
<td>237±30 (3)</td>
<td>0.013±0.001 (3)</td>
</tr>
<tr>
<td><strong>Mean values</strong></td>
<td><em><em>1.4±0.4</em> (22)</em>*</td>
<td><em><em>267±73</em> (19)</em>*</td>
<td><em><em>0.012±0.004</em> (22)</em>*</td>
</tr>
</tbody>
</table>

* Except **wt 3507** and **W303 gut1Δ**

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Non-induced cells
Exponentially growing
Glucose present
Catabolic repression

Non-induced cells
Pre-stationary
Glucose exhausted
Ethanol present
Partial derepression

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Cells under glucose repression

Derepressed cells

Induced cells

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Glycerol metabolic pathway in *S. cerevisiae*

**Fructose 1,6 biphosphate**

- **Dihydroxyacetone phosphate**
  - **G3PDH mit**
  - **Gut2**
  - **Gpd1**
  - **Gpd2**

- **Dihydroxyacetone**
  - **DK**
  - **ATP**
  - **ADP**
  - **NAD(P)H**
  - **NAD(P)**

- **Glycerol-3-phosphate**
  - **G3PDH**
  - **FADH2**
  - **FAD**
  - **NADH**
  - **NAD**

- **Glycerol**
  - **G3P**
  - **Gpp1**
  - **Gpp2**

**GDH**

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Glucose growing cells

Exponential growth phase
Simple diffusion

<table>
<thead>
<tr>
<th>Condition</th>
<th>Kd (M) ± Error</th>
<th>1 h⁻¹ g⁻¹ dry wt.</th>
<th>Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>No salt</td>
<td>0.005 ± 0.001</td>
<td>(5)</td>
<td>100%</td>
</tr>
<tr>
<td>1M NaCl</td>
<td>0.002 ± 0.0004</td>
<td>(3)</td>
<td>40%</td>
</tr>
</tbody>
</table>

Pre-stationary phase

Low affinity saturation kinetics
(Km ≈ 5mM)  Fps1...?
and
Simple diffusion

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# Ethanol grown cells

<table>
<thead>
<tr>
<th>Incubation in</th>
<th>Transport of $[^{14}C]$glycerol</th>
<th>Transport of H(^+) upon glycerol addition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$K_m$ (mM)</td>
<td>$V_{max}$ ($\mu$mol.h(^{-1})g d.wt(^{-1}))</td>
</tr>
<tr>
<td>No salt</td>
<td>1.14 ± 0.34</td>
<td>435.8 ± 21.6</td>
</tr>
<tr>
<td>1M NaCl</td>
<td>1.49 ± 0.78</td>
<td>428.9 ± 73.4</td>
</tr>
</tbody>
</table>

Lages and Lucas, 1997

It does not induce the transporter:

- To grow in MM glucose with 1M NaCl
- To grow in MM glucose and transfer to MM glucose + 1M NaCl

Lages and Lucas, 1997
**S. cerevisiae**

wt W303

Effect of the protonophore **CCCP** over the initial uptake rates of increasing glycerol concentrations

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**Relative velocity (%)**

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**[Glycerol] (mM)**

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100% - in the absence of **CCCP**

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Sutherland *et al.*, 1997

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\textit{S. cerevisiae}

THE STATE OF THE ART

Exponentially growing cells on ethanol

Exponentially growing cells on glucose

Glucose pre-stationary phase culture

\[ V / [\text{Glycerol}] (\text{l.h}^{-1} \text{ g}^{-1} \text{ dry wgt.}) \]

Lages and Lucas, 1997

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Glucose growing cells

Glycerol kinase activity

wt cells grown on ethanol: 61 (mU/mg protein)
wt cells grown on glycerol: 68 (mU/mg protein)
Comparing glycerol uptake V\text{max} \\
\textbf{Ethanol grown cells} \\

<table>
<thead>
<tr>
<th>Strain</th>
<th>V\text{max} (\mu\text{mol h}^{-1} \text{ g}^{-1} \text{ d.wt.})</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt (carrier + channel + glycerol kinase)</td>
<td>267\pm73 (19)</td>
<td>100</td>
</tr>
<tr>
<td>\textit{gup}\Delta (channel + glycerol kinase)</td>
<td>181\pm12 (3)*</td>
<td>\pm67</td>
</tr>
<tr>
<td>\textit{gup}\Delta \textit{fps1}\Delta (glycerol kinase)</td>
<td>137\pm10 (3)*</td>
<td>\pm51</td>
</tr>
<tr>
<td>\textit{gut1}\Delta (carrier + channel)</td>
<td>165\pm25 (3)</td>
<td>\pm62</td>
</tr>
<tr>
<td>\textit{gup}\Delta \textit{gut1}\Delta (channel)</td>
<td>\textbf{No uptake}*</td>
<td>0</td>
</tr>
</tbody>
</table>

\textbf{GUP - Glycerol Uptake Permease putative gene}

* Results obtained by B. H"{o}lst - Carlsberg Laboratory, Denmark

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Close collaboration with:

B. Hölst and M. Kielland-Brandt  
Carlsberg Laboratory  
Copenhagen, Denmark

Our thanks to:
• Lennart Adler and all the Göteborg group and  
• B. Rønnov from Danisco Laboratory, Denmark  
for supplying some of the mutants used in this work.

Financial support:  
EC Cell Factory Program - Project BIOTECH PL 950161

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