

Yeast orthologues associated with glycerol transport and metabolism

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Cloning orthologues of genes involved in glycerol transport and metabolism in *Saccharomyces cerevisiae*

GPD1 and *GPD2* (encoding glycerol 3-P dehydrogenase)
GUP1 and *GUP2* (involved in glycerol active uptake)
FPS1 (encoding the glycerol channel)
GUT1 (encoding glycerol kinase)

BLAST searches in:

Candida albicans Stanford Genome Database (URL: <http://genome-www.stanford.edu/Saccharomyces/>)
Génolevures program database (URL: <http://cbi.labri.fr/Genolevures/index.php>)

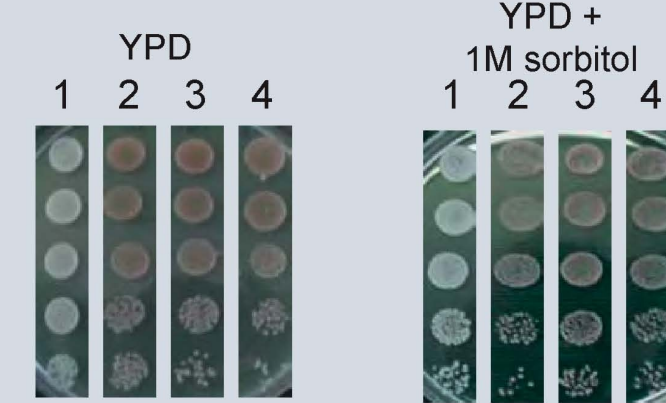
Orthologues cloned by primer walking:

Kluyveromyces thermotolerans *GPD1* Kt*GPD1*
Pichia angusta *GPD1* Pa*GPD1*
Candida tropicalis *GUP1* Ct*GUP1*
Pichia sorbitophila *GUP1* Ps*GUP1*
Candida albicans *GUP1* Ca*GUP1*
Kluyveromyces lactis *FPS1* Kf*FPS1*
Zygosaccharomyces rouxii *FPS1* Zr*FPS1*

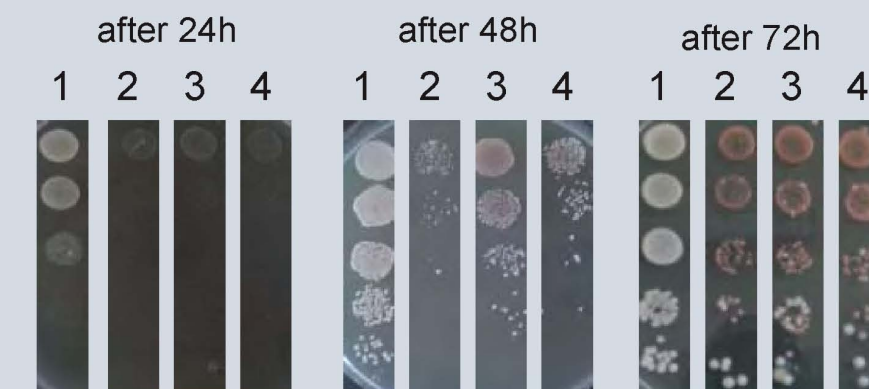
Orthologues cloned by RLM-RACE:

Kluyveromyces lactis *GUP1* Kf*GUP1*
Kluyveromyces marxianus *FPS1* Km*FPS1*
Pichia sorbitophila *GUT1* Ps*GUT1*

Complementation analysis for *FPS1* genes



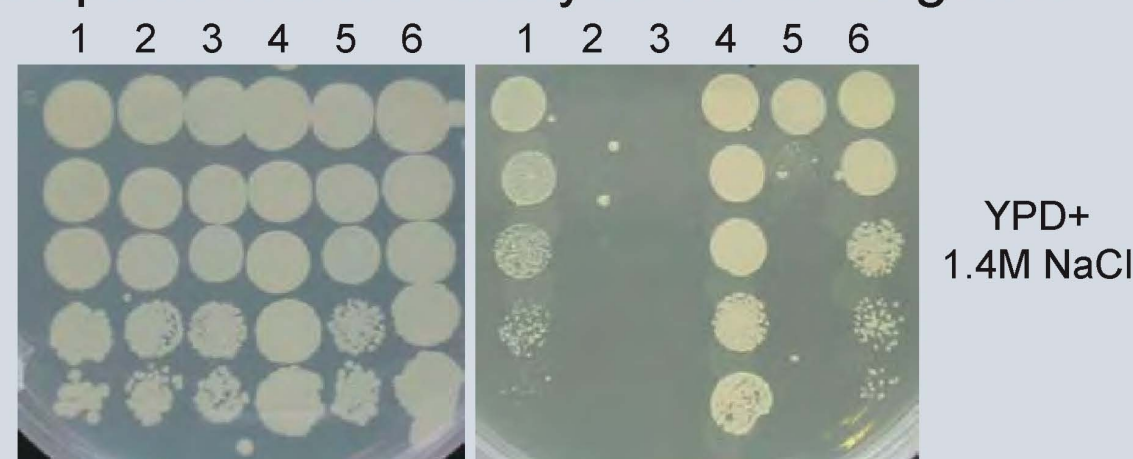
Hypoosmotic shock:
growth on liquid YPD + 1M sorbitol
and shift to YPD plates



- S. cerevisiae* W303-1A
- fps1* strain transformed with p426GPD (vector without insert)
- fps1* strain transformed with p416GPD with insert (Kf*FPS1*)
- fps1* strain transformed with p426GPD with insert (Zr*FPS1*)

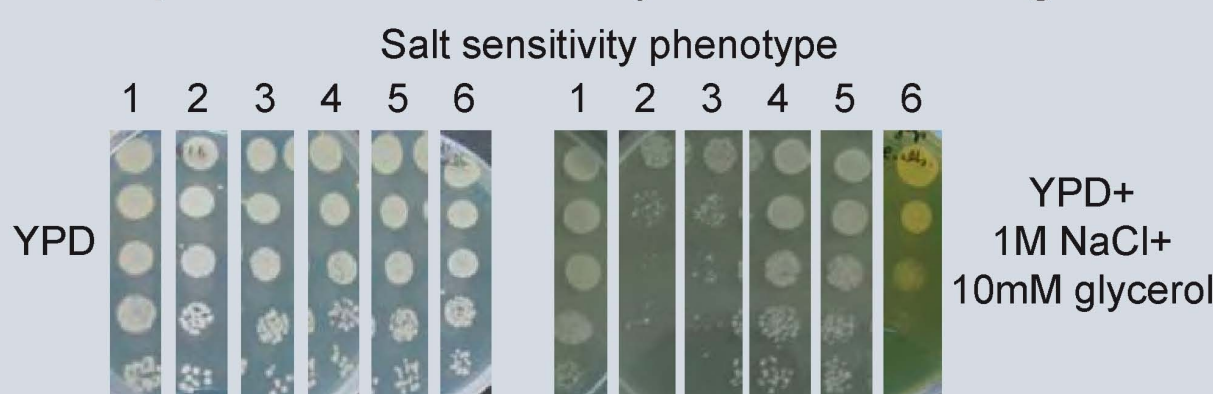
Cloned orthologues are able to complement *S. cerevisiae* mutants

Complementation analysis for *GPD* genes

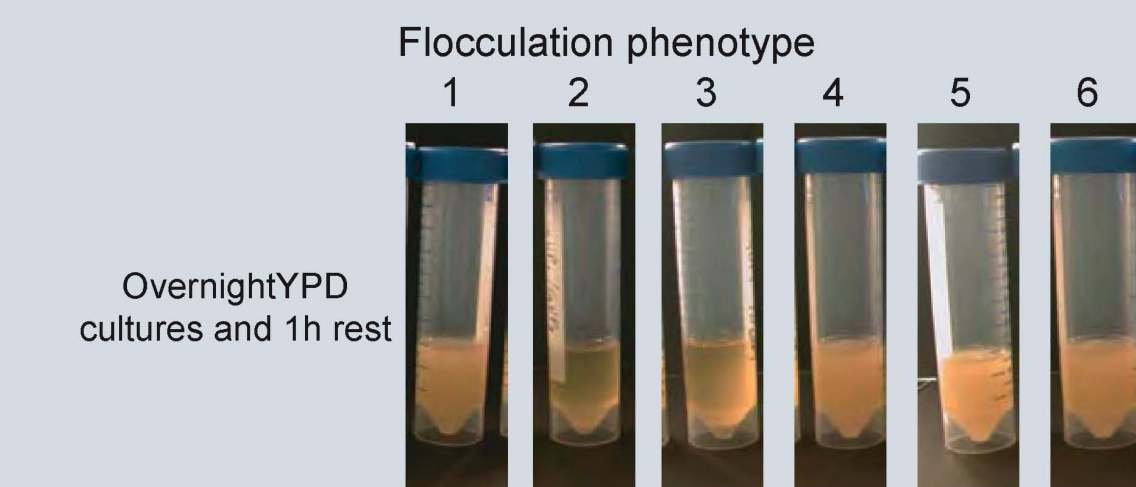


- S. cerevisiae* W303-1A
- gpd1gpd2* strain transformed with p423GPD (vector without insert)
- gpd1gpd2* strain transformed with p413GPD (vector without insert)
- gpd1gpd2* strain transformed with p423GPD with insert (Kt*GPD1*)
- gpd1gpd2* strain transformed with p413GPD with insert (Kt*GPD1*)
- gpd1gpd2* strain transformed with p423GPD with insert (Pa*GPD1*)

Complementation analysis for *GUP1* genes



- S. cerevisiae* W303-1A
- gup1* strain transformed with p416GPD (vector without insert)
- gup1* strain transformed with p426GPD (vector without insert)
- gup1* strain transformed with p416GPD with insert (Ps*GUP1*)
- gup1* strain transformed with p426GPD with insert (Ps*GUP1*)
- gup1* strain transformed with p426GPD with insert (Ca*GUP1*)

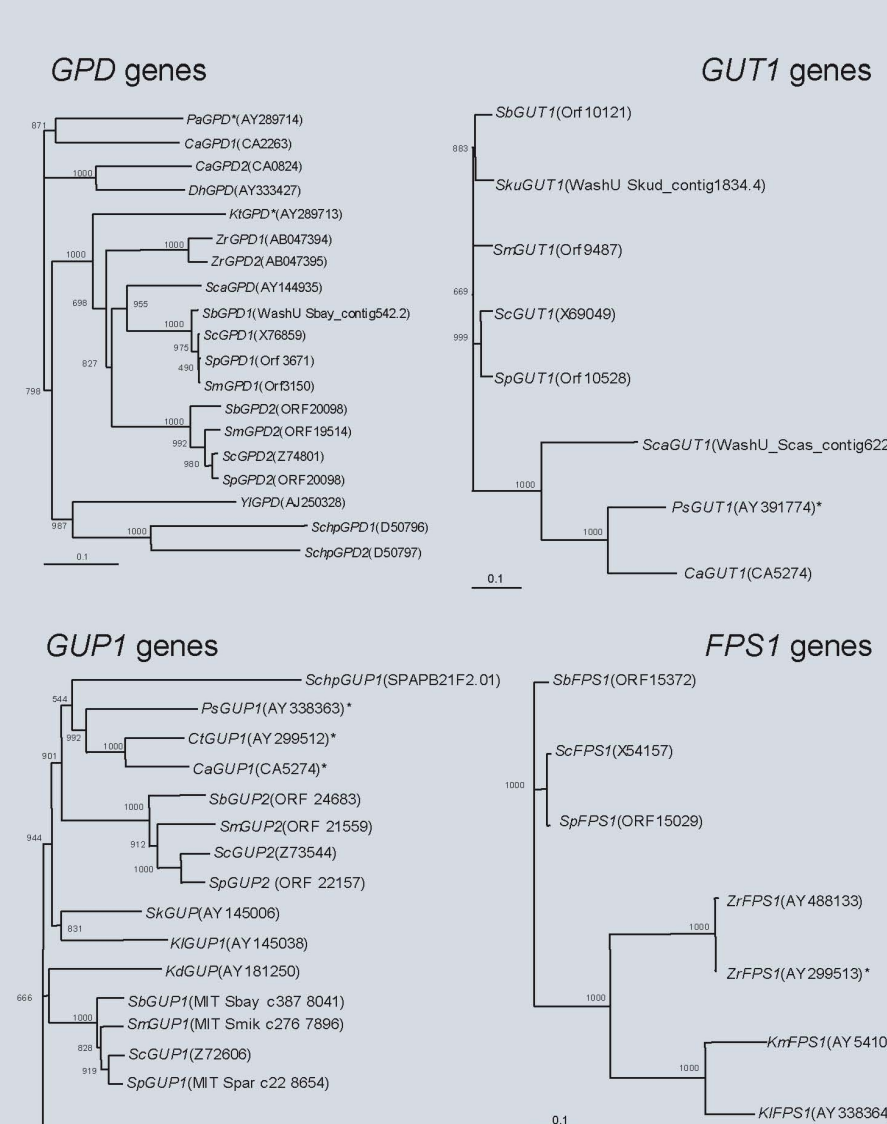
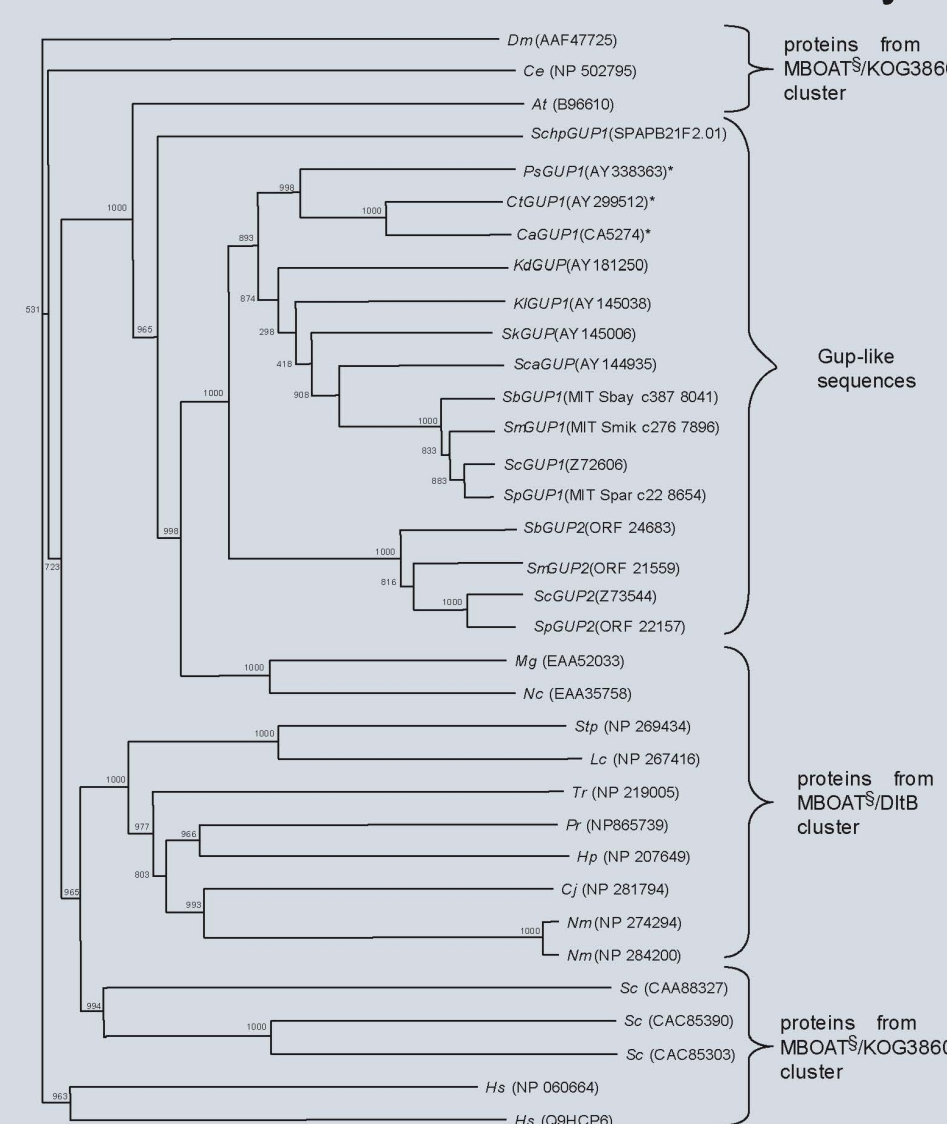


- S. cerevisiae* W303-1A
- gup1* strain transformed with p416GPD (vector without insert)
- gup1* strain transformed with p426GPD (vector without insert)
- gup1* strain transformed with p416GPD with insert (Ps*GUP1*)
- gup1* strain transformed with p426GPD with insert (Ps*GUP1*)
- gup1* strain transformed with p426GPD with insert (Ca*GUP1*)

Sequence homology analysis



GUP genes are most likely members of the MBOAT³ family



Phylogenies of the predicted amino acid sequences of the mentioned genes. Accession numbers are between brackets. *Genes cloned and tested for phenotypic complementation in this work.

- Organisms other than yeasts:
At *Arabidopsis thaliana*
Ce *Caenorhabditis elegans*
Cj *Campylobacter jejuni*
Dm *Drosophila melanogaster*
Hs *Homo sapiens*
Hm *Helicobacter pylori*
Hs *Homo sapiens*
Lc *Lactococcus lactis*
Mg *Magnaporthe oryzae*
Nm *Neurospora crassa*
Nm *Neurospora meningidis*
Pr *Pirene pygmaea*
Ssp *Schistosoma japonicum*
Tr *Trichostema pallidum*