NOVEL EVOLVED *YARROWIA LIPOLYTICA* STRAINS FOR ENHANCED GROWTH AND LIPID CONTENT UNDER HIGH CONCENTRATIONS OF CRUDE GLYCEROL

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Introduction

- Among the oleaginous yeasts, ***Yarrowia lipolytica*** appears to be a **single-cell oil producer** with great potential.
- **Crude glycerol**, derived from biodiesel industry, can be **valorized** as carbon source to develop a **sustainable process** for single-cell oil production.
- Adaptive laboratory evolution (**ALE**) is employed to **improve the fitness** of *Y. lipolytica* MUCL 28849 while the metabolism rewires under **high concentrations** of pure (**PG**) and crude glycerol (**CrG**).
- **Dry biomass** and **lipid** concentration of Evolved Strains (**ESts**) are evaluated and superior ESts are studied through **RNA sequencing**.

Strategy

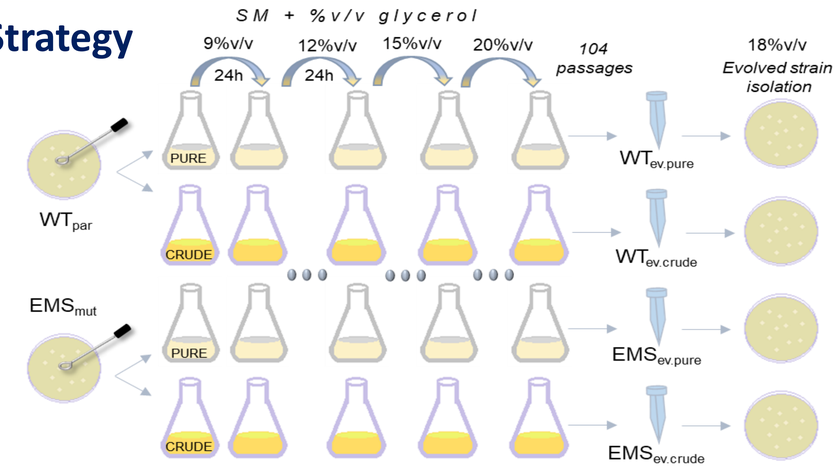
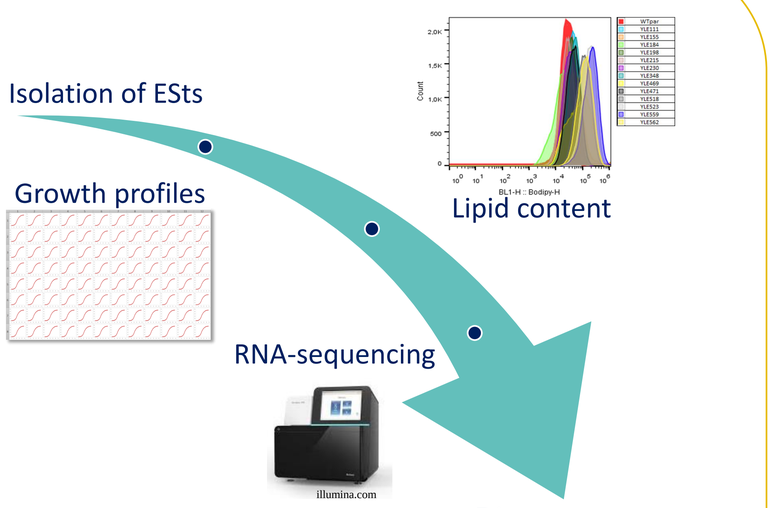


Figure 1. Schematic representation of ALE of the yeast *Yarrowia lipolytica*. Four ALE strategies were designed to increase biomass and lipid formation under high Pure Glycerol (PG) and Crude Glycerol (CrG) concentrations. Starting point: WT_{par}, *Y.lipolytica* 28849 parental strain; EMS_{mut}, after random chemical mutagenesis with methanesulfonate (EMS) exposure.



Results & Discussion

Monitoring of ALE

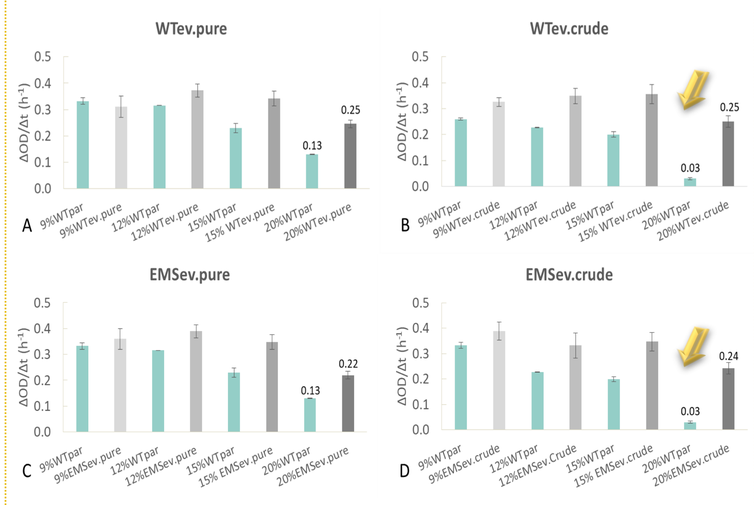


Figure 2. ΔOD/Δt rate during ALE experiment for increasing PG and CrG concentrations. Each graph corresponds to a different population (A. WTev.pure; B. WTev.crude, C. EMSev.pure; D. EMSev.crude); gray shades stand for different glycerol concentrations. Parental strain values are shown in green and error bars indicate standard deviation.

- **$\Delta OD_{600nm}/\Delta t$** rate of all Evolved populations (EPs) increased.
- **Stable phenotype** was reached for all EPs.

ESts Phenotype & Selection

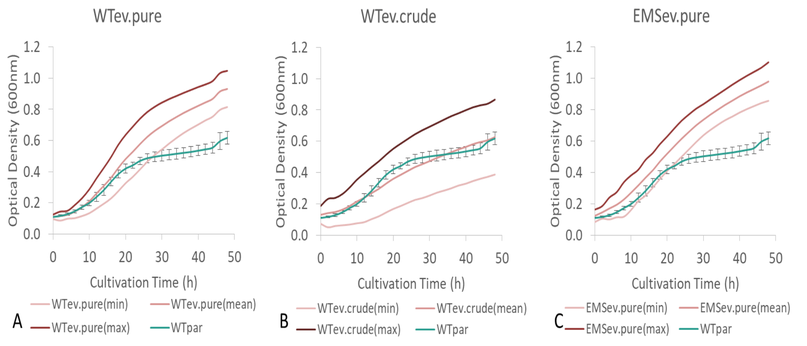


Figure 3. Growth profiles of **450** isolated ESts cultivated in 0.2 mL 15% v/v PG-Synthetic Medium (SM). Graphs depict the average, min and max growth curve of a. WTev.pure, n = 297; b. WTev.crude, n = 53; c. EMSev.pure, n = 100.

- ESts of $WT_{ev.pure}$ and $EMS_{ev.pure}$ showed from 1.5 to 2-fold higher biomass concentration at 48h, compared to WTpar.
- Growth profiling differences of ESts and cell observation through flow cytometry indicate the presence of heterogeneity.
- Multiple selection steps resulted in 5 superior ESts with enhanced biomass formation while lipid content was slightly increased.
- Culture of YLE155 (in 15%v/v CrG-SM) reached up to 2.4-fold increase of dry biomass in the first 24h.

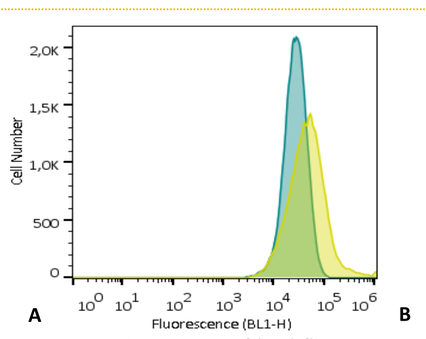


Figure 4. A. Comparison of lipid fluorescence and distribution per cell, between YLE155 (green) and WTpar (blue), using flow cytometry and bodipy dye (493/503nm), B. Growth profiles of **90** isolated ESts cultivated in 0.2 mL 15% v/v PG-SM. Graph depicts the average growth curve of 58 WTev.pure ESTs, 20 WTev.crude ESTs, and 12 EMSev.pure ESTs.

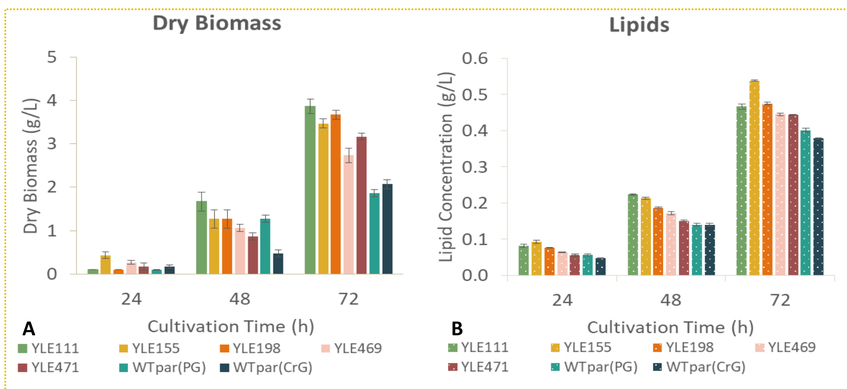


Figure 5. Observation of (A) dry biomass and (B) lipid concentration of **5** superior ESts, cultivated in 15% CrG-SM.

Differential Gene Expression

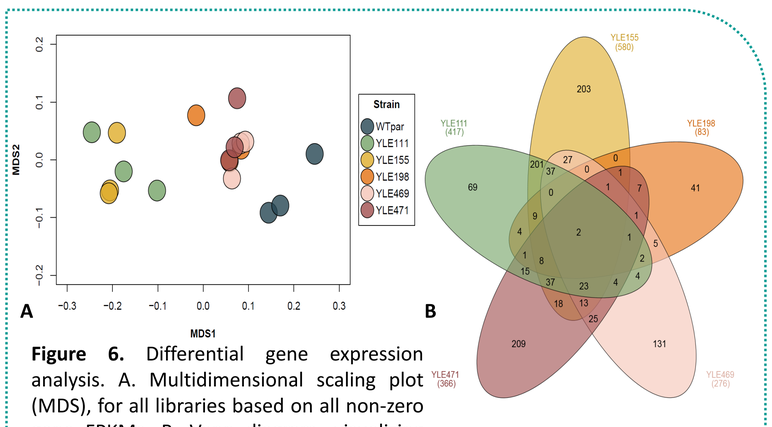


Figure 6. Differential gene expression analysis. A. Multidimensional scaling plot (MDS), for all libraries based on all non-zero gene FPKMs. B. Venn diagram, visualizing the overlap of differentially expressed genes among the different ESts.

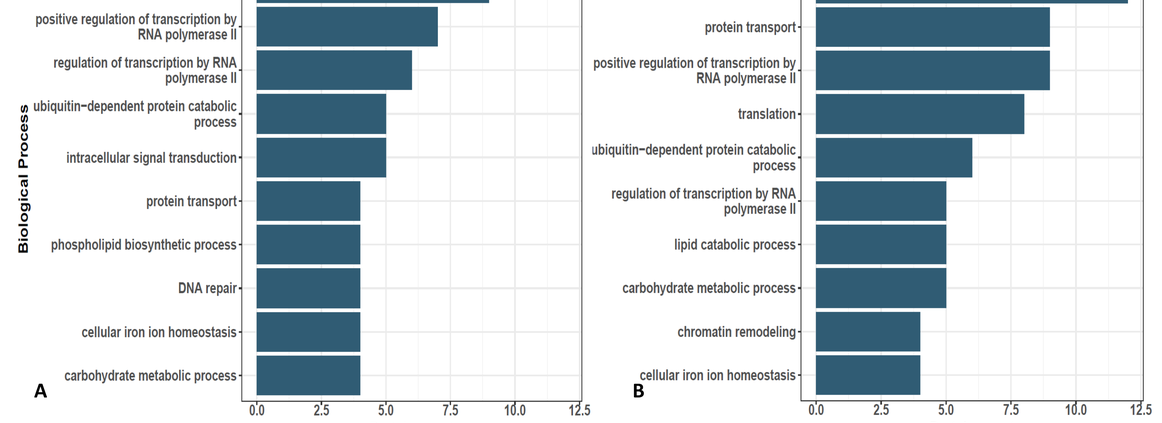


Figure 7. Abundance levels of the most abundant gene ontology (GO) - biological process (BP) terms associated with the differentially expressed proteins in A. YLE111 and B. YLE155.

- **Gene ontology annotation** of all 5 ESts (focused on BP) showed that most differentially expressed (**DE**) proteins were related to **membrane transport activation**.
- Transcriptome analysis revealed significant differential expression compared to WTpar, especially in YLE111 and YLE155 ESts with 417 and 580 DE genes, respectively.

Conclusions

- ✓ Utilization of crude glycerol was favored by applying ALE while growth profiles of ESts revealed phenotype enhancement in terms of biomass formation.
- ✓ Both flow cytometry and lipid concentration analysis verified that intracellular lipid levels of ESts were slightly increased.
- ✓ Initial changes in all derived ESts affected nucleosomal structure and regulation of transcription. In the more differentiated ESts, these changes globally affected membrane transport and protein transport processes.
- ✓ Gene ontology annotation analysis showed a similar trend in all ESts even though they originated from different ALE strategies.
- ✓ Fermentations in a lab scale bioreactor are currently conducted to determine biomass and lipid yields between selected ESTs and parental strain.

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