TRANSCRIPTOME VARIABILITY OF YEAST STRAINS

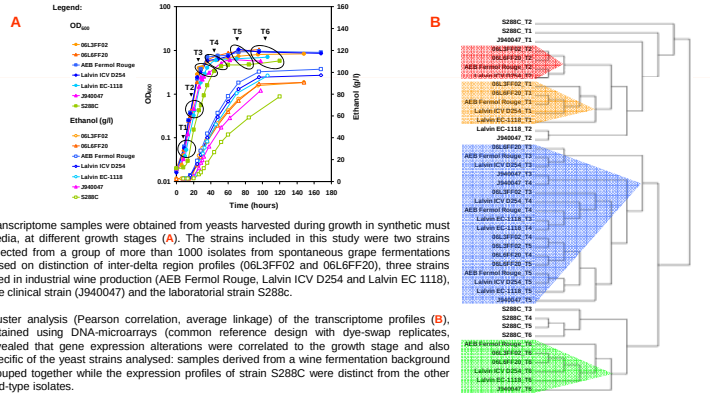
Laura Carreto[1], Dorit Schuller[2], and Manuel A.S. Santos[3]

[1]Biocant-Genomics Unit, Parque Tecnológico de Cantanhede, 3060-197 Cantanhede, Portugal; *[2]CBMA, Department of Biology, Campus de Gualtar, 4710 - 057 Braga*, Portugal; *[3]CESAM, Department of Biology, Universidade de Aveiro, Campus de Santiago, 3810-193 Aveiro, Portugal.* E-mail: msantos@bio.ua.pt

Introduction

Identifying genome and gene expression features associated with phenotypic variation is important to understand the mechanisms of adaptation to environmental change. We compared the genome and transcriptome of six wild-type *Saccharomyces cerevisiae* strains isolated from different biotopes and the laboratorial strain S288C, using oligo-DNA microarrays probing the yeast ORFeome. Comparative genome hybridization on array showed that only about 3 % of the ORFeome of the wild-type isolates had copy number alterations (mostly depletions) relatively to the laboratorial S288c strain (results not shown). Transcriptome profiling distinguished strains isolated from a wine fermentation background from the others (Figure 1) and showed high transcriptome variability in the late stationary growth phase (Figure 2), mostly due to the dramatic decrease of gene expression registered in wine fermenting strains (Figure 3). Transcriptome variability impacted different subsets of genes according to growth stage and affected key metabolic pathways associated to fermentation, among others (Figure 4).

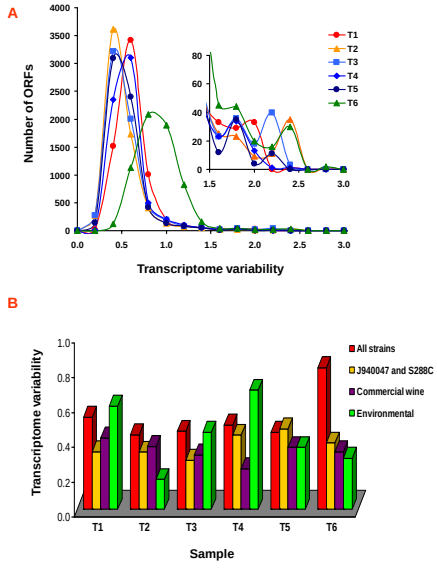
1. Transcriptome profiling during growth of different strains in synthetic must media



Transcriptome samples were obtained from yeasts harvested during growth in synthetic must media, at different growth stages (A). The strains included in this study were two strains selected from a group of more than 1000 isolates from spontaneous grape fermentations based on distinction of inter-delta region profiles (06L3FF02 and 06L6FF20), three strains used in industrial wine production (AEB Fermol Rouge, Lalvin ICV D254 and Lalvin EC 1118), one clinical strain (J940047) and the laboratorial strain S288c.

Cluster analysis (Pearson correlation, average linkage) of the transcriptome profiles (B), obtained using DNA-microarrays (common reference design with dye-swap replicates, revealed that gene expression alterations were correlated to the growth stage and also specific of the yeast strains analysed: samples derived from a wine fermentation background grouped together while the expression profiles of strain S288C were distinct from the other wild-type isolates.

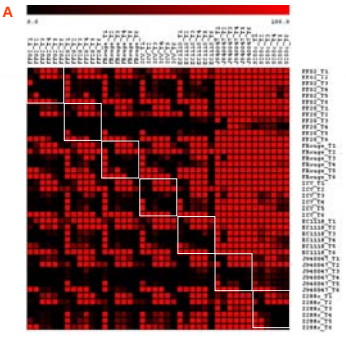
2. Transcriptome variability is higher in late stationary growth phase



Transcriptome variability between the analysed strains, measured as the average deviation of relative transcript abundance, was higher at the late stationary growth stage T6 (A). The normal distribution of the transcriptome variability tailed towards high variability values (see inset in graph A)

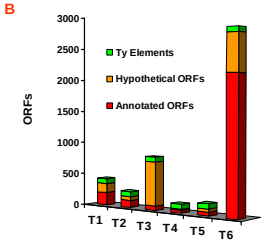
High variability in T6 samples was due to differences in gene expression between the groups of non-wine related strains (J940047 and S288c), commercial wine strains (AEB Fermol Rouge, Lalvin ICV D254 and Lalvin EC 1118) and the environmental wine strains (06L3FF02 and 06L6FF20), as indicated by the variability calculated for these sub-stets of strains (B). Environmental wine fermenting strains showed a high transcriptome variability in samples T1 and T4, during adaptation to new growth conditions in the beginning of exponential growth and of stationary growth phases, respectively.

3. Transcriptome alterations distinguish strains J940047 and S288C from wine fermenting strains



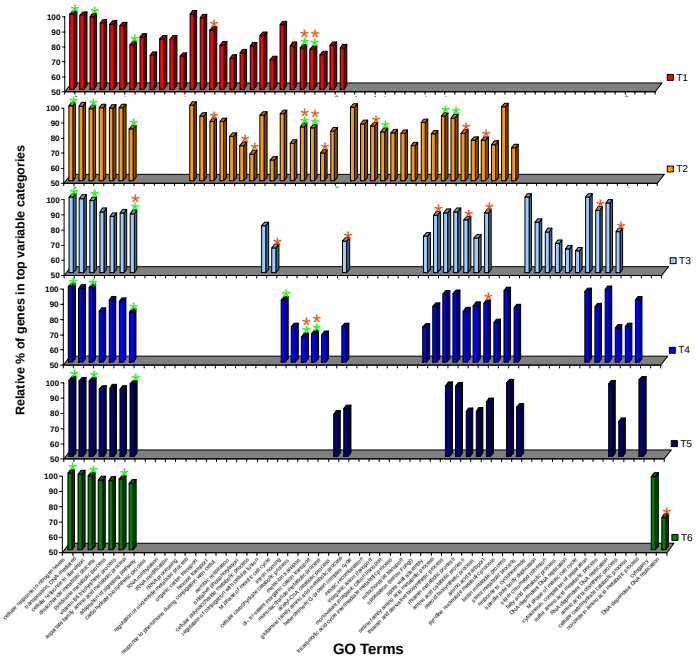
The correlation matrix (A) obtained by plotting the number of up regulated transcripts (SAM analysis) of one sample (in columns) relative to another (in rows) shows that strains J940047 and S288C distinguish from other strains adapted to wine fermentations in the extent of transcriptome alterations.

Differences between wine and non-wine strains are mostly due to decreased expression of many genes in wine strains. Transcript abundance decrease in wine fermentation related strains affects functionally annotated ORFs as well as Ty elements (B). At a late stage of growth (T6) the massive decrease in expression of the ORFeome impacts mainly on genes related to transcription and regulation of transcription. Down regulation in gene expression in other stages of growth affected genes involved in aspartate family and sulphur amino acid metabolism.



4. Transcriptome variability affects different functional categories according to growth stage

Functional categories of the most variable transcripts at the different growth phases were obtained using the FatiSCAN algorithm available in the BABELOMICS suit of annotation tools (http://babelomics.bioinfo.cipf.es/). The transcriptome list was ranked according to the average deviation value of the relative transcriptome M values and the abundance of genes in a given functional category was compared between the top and the bottom of the list. The graphic shows the functional categories (GO level 7) identified as more variable (relative percentage of genes in the top of the list as compared to the relative percentage in the bottom of the list) with a corrected *p*-value above 0.05.
Functional categories where average deviation was above 1.5 (see inset in Figure 3.A) were highlighted: (*) Highly variable categories with copy number depleted ORFs in wild-type strains relatively to the laboratorial strain S288c by comparative genome hybridization; (*) highly variable categories where ORF copy number alterations were not detected.



Conclusions

Variability in gene expression in *Sacchararomyces cerevisiae* strains was found in genes belonging to functional categories related to metabolic pathways central to survival and adaptation to changing environments, such as sugar and metal ion transport, nitrogen starvation and amino acid metabolism, throughout exponential and stationary growth stages. However, higher variability was found in late stationary growth, and this was mostly explained by repression of transcription of many genes in wine fermenting strains relatively to the others monitored, namely the clinical isolate J940047 and the laboratorial strain S288c. integration of comparative genome hybridization and the results from transcriptome profiling suggests that extensive changes in the global patterns of gene expression were associated to subtle, rather than to gross, genome alterations along with possible post-transcriptional gene expression regulation mechanisms.

Acknowledgments The Microarray facility is being supported by the Research Institute of the University of Aveiro (Project Ref: CTS 12), the Institute of Electronics and Telecommunications of the University of Aveiro (IEETA) and by FCT-FEDER through a re-equipment grant CONC-REEQ/737/2001. These studies were supported by FCT-FEDER grants POCI/SAU-MMO/55476/2004 and FEDER/POCI/BIA-PRO/55472/2004 and by Biocant Institute, Cantanhede, Portugal.