Iron homeostasis in *C. glabrata* at the omics level

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Iron is required for essential processes of cellular life, such as synthesis of DNA or respiration but is also toxic for living organisms because of production of free radicals. Tuning the iron uptake and metabolism is essential for the survival of pathogenic microorganisms in different host environments. Interfering with iron homeostasis might open new options for treatment of candidiasis which are still an important cause of high rate of morbidity and mortality. However data on iron homeostasis of pathogenic fungal agents, in particular *C. glabrata*, the second etiologic agent of candidiasis in United States and Europe, are very recent and limited. To enrich these data, we analyzed the phenotype and transcriptomic responses of *C. glabrata* CBS 138 strain and of its $\Delta aft1$ mutant to Fe²⁺ iron deficient and overload conditions.

Material and methods:

Strains: *C. glabrata* CBS 138 type strain

C. glabrata $\Delta aft1$ was obtained by deleting a 800 bp region containing the sequence of AFT 1 gene, using a PCR splicing method.

C. glabrata $\Delta aft1$ was complemented by using pyR 33 plasmid.

Growth conditions of CBS 138 and its $\Delta aft1$ mutant

Phenotype assays: YPD agar alone or supplemented with 100 μ M BPS (iron deficient), or supplemented with 100 μ M BPS and 100 μ M FeSO₄ (iron restored condition), at 30°C for 48h. Media at pH 4 and 8 were buffered.

Microarrays assays : Preculture in in liquid YPD at 30°C overnight then, for 3 hours at 30°C and 37°C, liquid YPD with 100 μ M BPS (2 iron deficient conditions) or liquid YPD with 500 μ M of FeSO₄ (2 iron overload conditions).

Results

Using “iron” keyword allowed to identify 130, 205 and 266 genes in *C. glabrata*, in *S. cerevisiae* and in *C. albicans* respectively. Addition of Heme, Fe, Carrier Proteins, Ferrous, Siderophore, Hemo, Transferrin and Hemin terms allowed find 12, 44 and 35 supplementary genes in *C. glabrata*, in *S. cerevisiae* and in *C. albicans*.

Transcriptomic analysis of the 142 genes identified by the GO terms in *C. glabrata* (Figure 2) confirmed the major role of AFT1 and FET3 and 4, FTR 1 and a GRX family gene (yellow square) in iron homeostasis.

636 genes of *C. glabrata* were differentially expressed (figure 3), 50 were within the 142.

Conclusion : *C. glabrata* iron homeostasis resembles more to that of *S. cerevisiae*. This is well illustrated with the major role played in *C. glabrata* by Aft1, the well-established regulator of *S. cerevisiae* iron homeostasis. It is particularly striking for FET4, FRE6, MRS3, MRX2, GRX family genes that are regulated by Aft1. Moreover *C. glabrata* misses Fe³⁺ transporters genes as the FIT family but exhibits interesting regulated genes not described as belonging to the iron regulon of *S. cerevisiae* or *C. albicans*.

Not surprisingly, the iron regulon of *C. glabrata* seems more related to its phylogenetic position closer to *S. cerevisiae* than to *C. albicans*, than to its rank of pathogenicity.

Omics

Genomics: Identification of candidate genes to be involved in iron homeostasis was performed by downloading Open Biomedical Ontologies files for *S. cerevisiae*, *C. albicans* and *C. glabrata* from Gene annotation from SGD, CGD, GO Consortium and GRYC databases. “Iron” keyword search was then completed with other terms search i.e. Heme, Fe, Carrier Proteins, Ferrous, Siderophore, Hemo, Transferrin and Hemin.

Functional characterization of the lists of genes was performed by using Biological process ontology then GO functional clusters were ordered into more general functional categories using a GO hierarchy.

Transcriptomics: Microarray assay

RNAs extracted with Quiagen RNAeasy minikit were hybridized on Agilent slides.

Microarray production (GE 8x15K, 60mer, 8 microarrays per slide) was performed by using Earray (Agilent) (<https://earray.chem.agilent.com>) and OligoArray

(<http://berry.engin.umich.edu/oligoarray/>). Results were analysed using ManGO for raw data normalization (<https://www.ncbi.nlm.nih.gov/pubmed/17586547>) and LIMMA

(<http://bioconductor.org/packages/release/bioc/html/limma.html>) for identification of differentially expressed genes.

A

B

Figure 2. Regulation of the 142 candidate genes involved in iron homeostasis, in *C. glabrata* and associated general functions. A. iron depleted. B. iron overload. Circles represent genes, colour and size is proportional with the level of expression. Rectangles represent cellular functions. Lines represent the links between genes and cellular functions.