

IDENTIFICATION OF GENES DIFFERENTIALLY EXPRESSED IN HYPHAE OF *CANDIDA ALBICANS*

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ABSTRACT

The ability to switch from yeast to hyphal forms is essential for *Candida albicans* virulence. This morphological switch involves the expression of hyphal-specific genes under the control of transcriptional factors. To contribute to the discovery of hyphal-specific genes, we used a differential screening method where clones of a genomic DNA library were hybridized with yeast and hyphal cDNA probes. Two clones with increased expression in hyphae were selected for study. Sequencing these clones, we found that they encoded two important metabolic genes, *CaHXT7* (high-affinity hexose transporter) and *CaYLL34* (member of the AAA ATPase family). *CaHXT7* and *CaYLL34* ORFs were completely determined. Analyses of the putative proteins show that: (1) CaHxt7p has one hexose transporter domain and (2) CaYll34p has two AAA ATPase domains. These results show, for the first time, increased expression of metabolic genes in *C. albicans* hyphae. Also, because the proteins encoded by *CaHXT7* and *CaYLL34* may be necessary for the switching to hyphae, they could be new targets for antifungal drugs.

Key words: Differential screening, *Candida albicans*, HXT7, YLL34.

INTRODUCTION

The yeast *Candida albicans* is an opportunistic pathogen of humans. Usually, it is commensal and colonizes the mucosal surfaces of the gastrointestinal tract although certain factors, such as host immune-deficiency, may trigger life-threatening disseminated infections (4).

C. albicans can grow in a variety of morphological forms, ranging from budding yeast to pseudohyphae and hyphae, where the latter is a more pathogenic form. Different signaling pathways and transcriptional factors seem to converge to regulate the transcription of a common set of hyphal-specific genes (3). Several genes have been identified whose expression is induced during hyphal growth and most of them encode cell-wall proteins. Probably, most of hyphal-specific genes have

not yet been identified. Therefore, we used a prospective method, the differential screening, to identify differentially expressed genes in hyphae of *C. albicans*.

MATERIALS AND METHODS

To obtain *Candida albicans* genome library, genome DNA of *Candida albicans* ATCC 90029 was extracted and submitted to ultrasound treatment. The fragments from 1 to 4 kb were selected and cloned. Plasmids were extracted using "CottonPrep" method, performed in a 96 wells plate using a modified MiniPrep protocol.

C. albicans was grown in YPD media at 30°C to produce yeast cells. To obtain hyphal form, yeast cells were diluted in fetal bovine serum containing dextrose and incubated at 37°C

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for 3 hours. The total RNA was extracted and cDNA of yeast and hyphae were produced.

In differential screening assay, plasmids were plotted in a Dot Blot membrane. The membrane was hybridized with yeast and hyphae cDNA probes labeled with [α -³²P] dCTP. The images were analyzed by ImageQuant (Molecular Dynamics) program. The clones of interest were sequenced. As the inserts were greater than 2,000 bp, the plasmids were fragmented by ultrasound treatment. The fragments between 300 and 1000 bp were cloned. The inserts were sequenced and the contigs were assembled in Phred Phrap Consed program. The alignments were performed with the MEGALIGN program (DNASTar, Madison, Wisconsin) according to the Clustal method.

RESULTS

In order to identify genes that are up-regulated during the yeast-to-hypha transition, we used a differential screening method where a dot blot membrane containing clones of *C. albicans* library were hybridized with total cDNA probes of yeast and hyphal form (Fig. 1). The arrows show two clones (G3 and E4) that contain genes with increased expression in hyphal form. When compared with yeast expression, G3 and E4 clones were 1.7 and 3.1 times increased in hyphae, respectively.

Sequencing of these clones, we found that they encoded two important metabolic genes, *CaHXT7* (high-affinity hexose transporter) and *CaYLL34* (member of the AAA ATPase family).

The sequence of each gene was aligned to the homologous in *S. cerevisiae* and contig of Candida Genome database in order to determine the complete ORF (Figs. 2 and 3). To find the ORFs of contigs G3 and E4, these sequences were submitted to “Find ORF” tool in NCBI site.

CaHXT ORF encode a putative protein of 540 amino acids. Analyzing CaHxt7p on pfam site, a hexose transport domain was found. *CaYLL34* ORF encodes a protein of 828 amino acids and on pfam analysis, it presented two domains common to the AAA ATPase family.

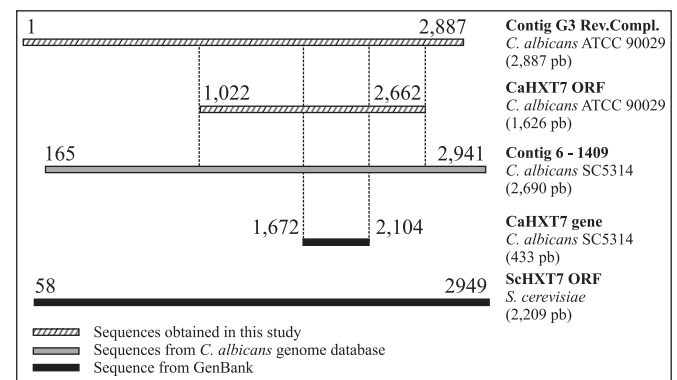


Figure 2. Diagram of alignment of *HXT7* genes sequences. These sequences were obtained from two strains: *C. albicans* ATCC 90029 (this study) and *C. albicans* SC5314 (*Candida* Stanford Genome).

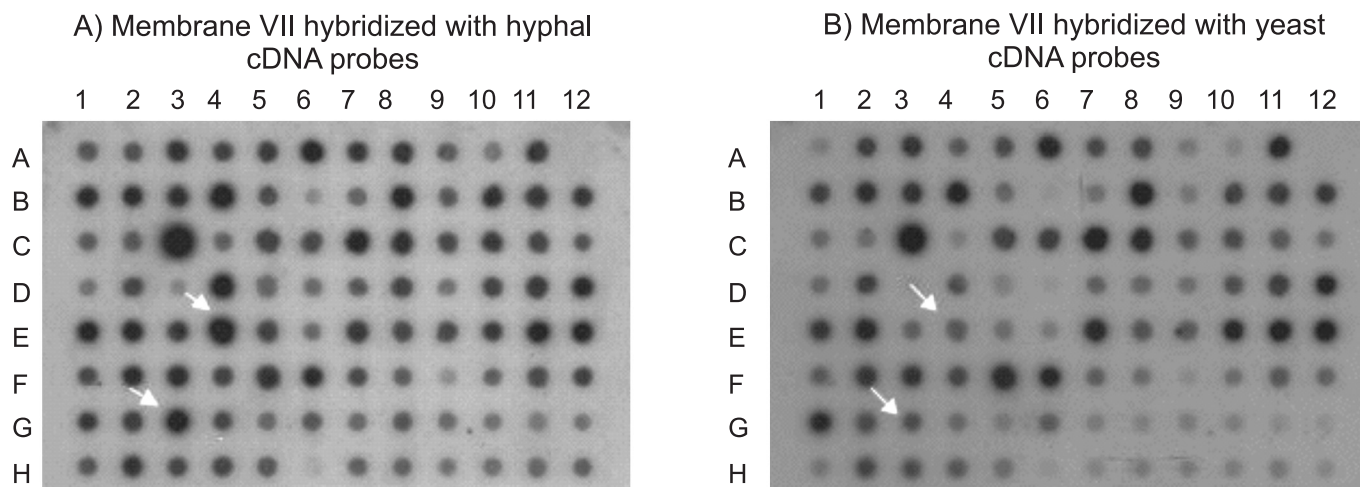


Figure 1. Differential Hybridization. Dot Blot of membrane VII hybridized with hyphae cDNA probes (A) and yeast cDNA probes (B). Each position contains plasmids of *C. albicans* ATCC 90029 genomic library. A1, D6, H1 and H12 positions contains plasmids from *C. Albicans* actin gene clone, used as positive control and signal normalization. A12 position contains no plasmid, used as reaction blanc. G12 position contains pUC18 plasmid without insert, used as negative control. The arrows indicate the clones with the genes with increased expression in hyphal form (positions E4 and G3).

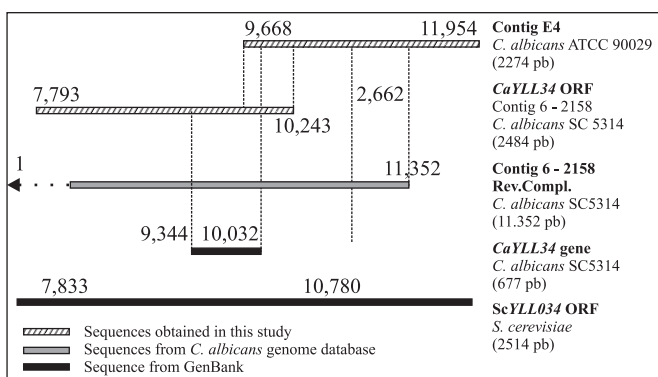


Figure 3. Diagram of alignment of *YLL34* genes sequences. These sequences were obtained from two strains: *C. albicans* ATCC 90029 (this study) and *C. albicans* SC5314 (*Candida* Stanford Genome).

DISCUSSION

Our results suggest that genes *CaHxt7* and *Hxt7* (*S. cerevisiae*) are homologous based on their sequence similarity (58.8%). In *S. cerevisiae* this gene encodes protein *Hxt7*, a high-affinity glucose transporter (1). The increased expression of *CaHXT7* in *C. albicans* hyphae could be a response to the low glucose concentration in the microenvironment during tissue invasion.

The ORF *CaYLL34* has high sequence similarity to ORF *YLL034c* of *S. cerevisiae* (58.3%) suggesting that they are homologous. *YLL034c* of *S. cerevisiae* is essential for cell growth (2). *CaYLL34*, as a member of the AAA ATPase family, is probably necessary for membrane fusion and protein degradation. Hyphae of *C. albicans* have a great number of vacuoles that contain proteinases necessary to tissue invasion and *CaYll34p* may have an important role in their fusion and Ub-Pr degradation.

This is the first study reporting the up-regulation of metabolic genes during yeast-hyphae transition. Also because the proteins encoded by these genes may be necessary for the switching yeast to hyphal form, they can be new targets for the development of antifungal drugs.

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RESUMO

Identificação de gases em hifa de *Candida albicans*

A transição morfológica de levedura para hifa é essencial para a virulência de *Candida albicans*. Esta transição envolve a expressão de genes hifa-específicos que estão sob o controle de fatores transcricionais. Para descobrir genes hifa-específicos utilizamos um método de triagem diferencial, onde clones de biblioteca de DNA genômico foram hibridizados com sondas de cDNA de levedura e hifa. Dois clones com aumento de expressão em hifa foram selecionados. O sequenciamento dos insertos destes clones permitiu a identificação de dois genes metabólicos importantes: *CaHXT7* (high-affinity hexose transporter) e *CaYLL34* (da família AAA ATPase). As ORFs completas destes genes foram caracterizadas e a análise das proteínas hipotéticas revelou que: (1) *CaHxt7p* tem um domínio de transportador de hexose e (2) *CaYll34* tem dois domínios AAAATPase. Este é o primeiro estudo que demonstra aumento de expressão de genes metabólicos em hifas de *C. albicans*. Ainda, a associação dos produtos de *CaHXT7* e *CaYLL34* com a formação de hifas torna estas proteínas potenciais novos alvos para drogas antifúngicas.

Palavras-chave: triagem diferencial, *Candida albicans*, HXT7, YLL34.

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