Structural and Functional Peculiarities of Outer and Central Cell Layers of Yeast Colony

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ABSTRACT

Yeasts can develop multicellular structures on the solid surface, one of which is a colony consisting of two different cell groups, outer and central. In the present study genome variability and expression of *ato1* and *flo11* genes were compared between outer and central cell layers. Results revealed differences in transcriptional profiles between two cell groups and there was not observed any changes in microsatellite distribution in yeast genome.

Keywords: Gene expression, ISSR-PCR, yeasts colony differentiation.

INTRODUCTION

Adaptation to a wide range of various environments predetermines the diversity of morphological forms of yeasts as well as the heterogeneity of the cell populations inside multicellular structures, such as colonies, biofilms, filaments and others. Cell differentiation in yeast colony leads to the formation of cell groups possessing different functions: some of them produce nutrients, and others consume them [1]. The variety of the properties between cell groups is based on the activation of adaptive capabilities that have already been encoded by genome. Yeast colony was characterized as multicellular structure consisting of two cell groups — central and outer, differing by morphological, physiological, biochemical and metabolic features [2]. The cooperation between these cell groups provides the beneficial existence of the multicellular structure as an entire formation. Such multicellular structure has several advantageous features: more efficient use of nutrients, increasing resistance to stress.

It was postulated that the process of yeast colony formation consists of three development phases: 1st acidic, alkaline, 2nd acidic [2]. The switch from one phase to another was associated with pH changing and synthesis of signal molecules (as $NH4^+$), some transcriptional and metabolic changes was also observed.

OBJECTIVES

The purpose of the present research was to study and compare some structural and functional peculiarities of central and outer cell layers in yeast colony at the end of alkaline phase.

METHODOLOGY

Three yeast strains *S.cerevisiae* BY4743, *S.cerevisiae* UCM Y-524 and *Kluyveromyces marxianus* UCM Y-305 were cultivated on YEPD agar plates for 14 days at +28°C. The samples of central and outer cell layers were picked up with tooth sticks and frozen at -20°C before processing. Genomic DNA isolation and amplification with primers to short nucleotide repeats ($(GA)_9C$; $(AC)_8C$; $(AG)_8$) were carried out as described in [3]. RNA was isolated with Trizol reagent, RT-qPCR was performed on qTower 2.2 thermal cycler (Analytik Jena AG, Germany). Relative gene activity was calculated according to [4] and expression of t*af10* gene was used as endogenous control.

RESULTS

In our study yeasts were grown on YEPD agar plates for 14 days and any differences in growth rate between strains were not detected. But it was observed the specific colonies morphology for each yeast strain (Figure **1**): *S. cerevisiae* BY4743 and *K. marxianus* UCM Y-305 colonies were smooth but *S. cerevisiae* UCM Y-524 had lacy architecture.

Figure 1. Colonies of yeasts: A — *S. cerevisiae* BY4743, B — *S. cerevisiae* UCM Y-524, C — *Kluyveromyces marxianus* UCM Y-305.

Short nucleotide repeats are ubiquitous in yeast genomes and allow analyzing DNA variability caused by abiotic and biotic factors. Results of amplification with primers to microsatellite repeats revealed no differences in amplicon patterns. Totally, the size of PCR products varied from 200 to 2300 bp and the average number of bands was 6.

Metabolic heterogeneity of cell populations may be due to different transcriptional activity of genes. To verify this statement a comparative analysis of the relative expression of genes encoding transport protein responsible for the transport of $NH4^+$ (*ato1*) and glycoprotein involved in the flocculation, biofilm formation and invasive growth (*flo11*) was carried out. The results showed that in both *S. cerevisiae* strains expression of *ato1* gene was higher in outer cell layer while *flo11* activity increased in central region. The opposite profile of transcriptional activity of *ato1* and *flo11* genes was defined in *K. marxianus*: the level of *ato1* expression was risen in central colony zone and *flo11* – in outer zone (Figure **2**).

CONCLUSION

Thus, results of the present research revealed no genome variability but showed differences in transcriptional activity of *ato1* and *flo11* genes between outer and central cell groups in yeast colony and the mode of these differences was depended on the yeast species.

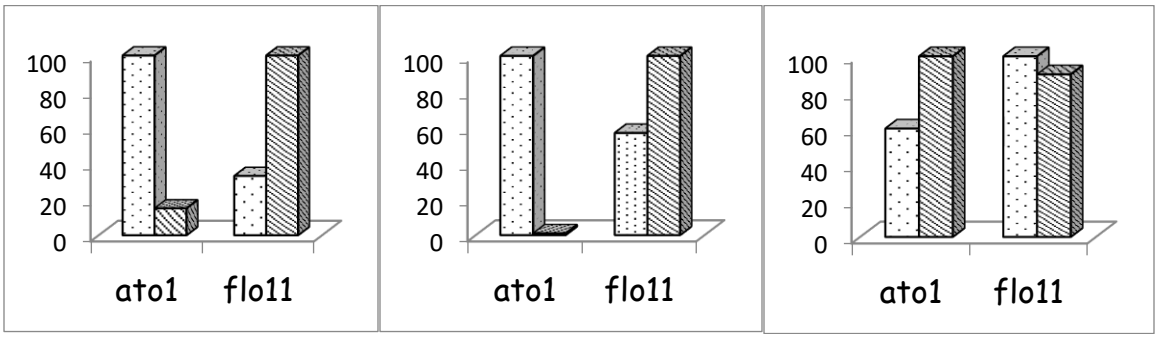

Figure 2. Differences of *ato1* and *flo11* gene expression between outer and central cell layers of yeast colony. A – *S. cerevisiae* BY4743, B – *S. cerevisiae* UCM Y-524, C – *K. marxianus* UCM Y-305.

outer cell layer, central cell layer.

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