under Stress Conditions through Image Processing

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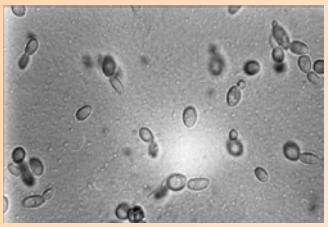
Yarrowia lipolytica is an aerobic microrganism capable to produce important metabolites, has an intense secretory activity which drives efforts to be employed in industry (as a biocatalyst), in molecular biology and genetics studies. Dimorphism is refeered to fungi ability to growth in two distinct forms, usually as single oval cells os as a filament and to be reversible between each one. The cell shape is controlled by environmental factors and has been seeked by some authors.

Y. lipolytica has been considered an adequate model for dimorphism studies in yeasts since it has an efficient system for transformation and is easy to distinct between its morphological forms, on opposite to *S. cerevisiae* that do not produce true filaments and exhibits pseudo-hyphae growth under nitrogen limited conditions. *Y. lipolytica* has an hyphae diameter corresponding 60 to 100% of its single cell stage [4,5]. It is believed that *Y. lipolytica* dimorphism is related to defense mechanism from adverse conditions.

The aim of this work resides on investigate morphological changes in *Y. lipolytica* under thermal and oxidative stress conditions. Both stress conditions were applied at exponential growth phase. Morphology was observed in a optic microscope (Axiolab, Zeiss) and cell characteristics were determined employing image processing analysis (Matlab v. 6.1, The Mathworks Inc.) and comparisons were carried on to a control system. Typical figures obtained for morphological analysis were presented below.

A net increase around 25% on hyphae formation was detected as well as a significant increment in its length in relation to control system, when both thermal and oxidative stress was applied. The results herein obtained drives to consider a possible relationship between dimorphism and a cell response mechanism to stress conditions.

Image Analysis Procedure



Green channel RGB image

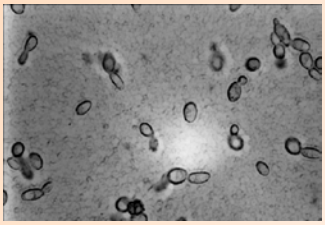


Image after a combination of bottom and top hat filtering

Image after binarization and morphological operations

Individual Properties:

❖ *Area* is the area of the projected surface of the object on the plane of vision.

❖ *Hyphal Length* and *Hyphal Width* were determined as the Maximum Feret diameter (F_{Max}) and Minimum Feret diameter (F_{Min}), respectively.

❖ *Elongation* is given by the ratio between *Hyphal Length* and *Hyphal Width* (F_{max} / F_{min})

Experimental Approach

•***Microorganism:*** A wild type strain of *Yarrowia lipolytica* (IMUFRJ 50682) was selected from an estuary in the vicinity of Rio de Janeiro, Brazil

• ***Media Composition (w/w):*** Glucose 2 %, peptone 0.64%, yeast extract 1%.

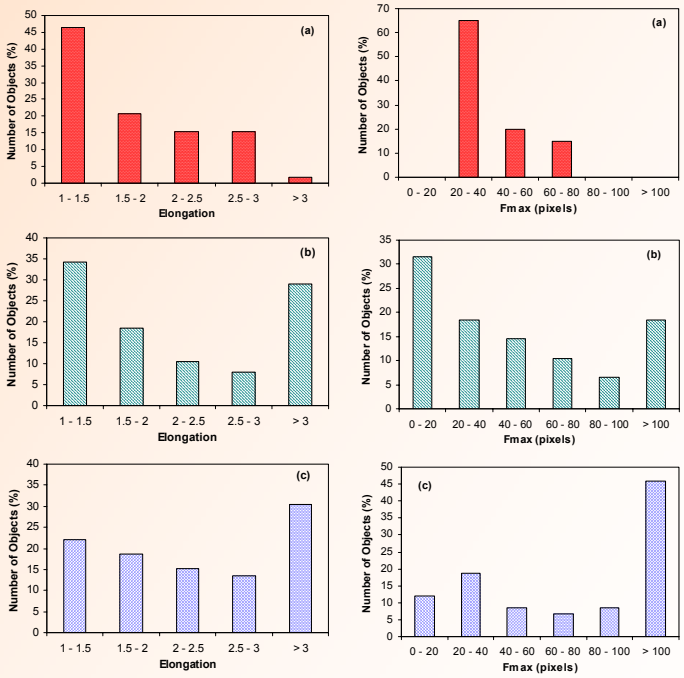
• ***Inoculum and Growth Conditions:*** 29^{0}C in a rotary shaker (160 rpm).

• ***Oxidative Stress:*** During exponential growth phase, an aliquot of hydrogen peroxide (H_2O_2) 1M was dropped into the medium, in the way to reach the desirable concentration (10 mM).

• ***Heat-Shock:*** The temperature shift was applied during the exponential and stationary phase, turning 29^{o}C to 37^{o}C during 1 hour.

Results

Based on Elongation factor: ***Based on Hyphal Length:***



(a) control system, (b) thermal stress and (c) oxidative one

Conclusions

Cell exposition to both stress conditions conducts to an increase in *Y. lipolytica* capacity of forming hyphae. The figures above denote the presence of intermediate morphological forms in conjunction with hyphae and unicellular ones.

For control system an usual gaussian size distribution was obtained. But, considering the stress systems, this characteristic behaviour is no longer observed, leading to a representative percentage of cells with high area values. Unfortunately, this parameter may not be adequate for dimorphism studies since cells shifted to a different morphological structure.

Although in both stress cases is possible to attest an increase in hyphae characteristic length, an oxidative condition permited to achieve higher values when compared to thermal ones.

Acknowledgments

CNPq Conselho Nacional de Desenvolvimento Científico e Tecnológico

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CAPES