TITLE: Influence of dimethylsulfoxide on lipase activity of *Yarrowia lipolytica*

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ABSTRACT: *Yarrowia lipolytica* is the most studied specie among the group of unconventional yeasts. It is known for producing and secreting various enzymes such as proteases, lipases, esterases and phosphatases, all of great biotechnological interest. *Y. lipolytica* has the cell wall with high hydrophobicity, which allows this species to consume hydrophobic substrates through different metabolic pathways in different cellular organelles. For this purpose, lipolytic enzymes carry out the hydrolysis of some of these hydrophobic substrates, mainly triglycerides, allowing the products of hydrolysis to enter in the cell. Encoded by the LIP2 gene the extracellular enzyme lip2p is the main extracellular lipase of *Y. lipolytica*. Considering the importance of this enzymatic class for industrial sectors, such as cleaning products, drugs and textiles, some strategies that aim at the highest yield in the production of this enzyme is necessary. The aprotic solvent dimethylsulfoxide, widely used in the pharmaceutical industry, due to the action of active carriers, is also used for cellular cryopreserving, as it replaces the water molecules, without generating irreversible conformational changes to the cellular components, besides increasing the flexibility of the membrane. Therefore, the present work proposes to evaluate the influence of DMSO on the extracellular enzymatic activity of *Yarrowia lipolytica*. For this, microdilution assays were performed based on the standard international CLSI / NCCLS (Clinical and Laboratory Standards Institute) methodology for yeasts (CLSI / NCCLS Standards M27-A2), in order to verify the minimum inhibitory concentration in the presence of dimethylsulfoxide (DMSO). *Y. lipolytica* cultures were then harvested for 96 h at 28 °C and 250 rpm in a 500 mL erlenmeyer flask containing 200 mL of YPD culture medium (1% yeast extract, 0.64% peptone and 2% % glucose), in the presence and absence of DMSO (minimal inhibitory concentration). It was possible to observe that from 5% of DMSO there was reduction in the cellular growth of *Y. lipolytica*. The minimal inhibitory concentration of DMSO (5%) when added to the *Y. lipolytica* culture significantly favored the activity of measured extracellular lipase. Thus, the use of the DMSO aprotic solvent represents an alternative to increase extracellular enzymatic activity in *Y. lipolytica* cultures.

Keywords: *Yarrowia lipolytica*, lipase, dimethyl sulfoxide.