TITLE: MYCELIUM-BOUND LIPASES PRODUCTION OF *Rhizopus Oryzae*: UTILIZATION OF FOOD INDUSTRY WASTE AS LOW-COST SUBSTRATES.

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ABSTRACT:

The production of lipases is an area of biotechnology in constant expansion, moving billions of dollars in the current market, and can be applied in different industrial segments such as detergents, textiles, cosmetics and pharmaceuticals. The composition of the culture medium must provide nutrients necessary for the growth of the microorganism, as well as induce the production of enzymes. Many substrates used for the production of lipases are expensive, the study of the best use of agro-industrial residues as a substrate in the fermentation process is vital to enable the application of these enzymes in industrial processes. Different low-cost substrates have been tested for the production of lipases such as vegetable oils, agro-industrial residues, poultry fats and tallow. Lipases produced by microorganisms can be extracellular or intracellular lipases. Among the intracellular lipases there are lipases bonded to the mycelium, which are still active, and therefore can be used as a biocatalyst in bioreactions, partially eliminating high-cost steps such as purification, recovery and immobilization. One of the fungi already reported as good producers of mycelium-bound lipase is the filamentous fungus *Rhizopus oryzae*. In this context, in the present study, whey was used as substrate, which contains in its composition the nutrients and lipids necessary for the growth and production of lipase of the fungus *Rhizopus oryzae* CCT3759. The effect of the addition of lipid inducers in the cultivation was also evaluated, such as olive oil, coconut oil and residual frying oil (Soybean). For that, submerged fermentations were carried out for 72 h at 30 ºC and 180 rpm of agitation, and at the end of each cultivation, the culture medium was vacuum filtered, and the biomass washed with water and acetone. The biomass obtained was evaluated for hydrolytic activity (U/g), biomass concentration (g/L) and total activity (U/L). In 72 h of cultivation, values of 167.06 ± 6.29 to 252.69 ± 14.43 U/g of hydrolytic activity, biomass concentration of 2.70 to 9.80 ag/L and total biomass activity were obtained from 451.06 to 2475.93 U/L. The results demonstrate that the whey was able to produce whole cells with satisfactory lipolytic activity (167.06 ± 6.29 U/g; 2.7 g/L; 451.06 U/L). However, the addition of lipid compounds as inducers was necessary to obtain a catalytic biomass with a greater hydrolytic activity, and the frying waste oil was the most efficient in promoting the formation of biomass and the expression of lipase bound to the mycelium, reaching the values of 252.69 ± 14.43 U/g, 9.8 g/L of biomass concentration and 2475.93 U/L of total biomass activity. Therefore, whey proved to be an efficient substrate for the production of whole cells of *Rhizopus oryzae* CCT3759 with lipolytic activity and the supplementation of whey with lipid inducers promoted a greater hydrolytic activity to biomass. Thus, residues such as whey and frying waste oil can be applied as feedstock in the production of lipases that can be applied in various reactions to produce high value-added products.

Keywords: *Rhizopus oryzae*, Whole-cells, Whey, Frying Waste Oil, Vegetable oils.