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Abstract

The *Yarrowia lipolytica* yeast is an oleaginous microorganism known for its ability to synthesise valuable metabolites with applications in the food, cosmetic, pharmaceutical and fuel industries. These yeasts are capable of assimilating a wide range of different carbon sources needed for growth and synthesis of desired compounds, such as hydrophilic (sugars, glycerol) and hydrophobic (hydrocarbons, fats) substrates, which can also be extracted from waste materials. This dissertation focuses on the study of the ability of the *Y. lipolytica* to synthesise large quantities of phospholipids, among which the main focus is on phosphatidylcholine, and resveratrol, a polyphenolic compound with proven health-promoting properties, using a low-cost substrate such as glycerol. The work also has an interdisciplinary aspect, with a focus on obtaining lipid derivatives of resveratrol and evaluating their anticancer and antioxidant properties against cancerous and healthy human cell lines.

The first phase of the study was undertaken to characterise the phospholipid biosynthetic pathway in the yeast *Y. lipolytica* and to enhance its production from a waste substrate, glycerol. For this purpose, a series of genetic manipulations were performed obtaining a strain (PS08) characterised by a 6-fold increase in the production of phosphatidylcholine (27.8 mg/g) and an almost 3-fold improvement in the production of total phospholipids (60.2 mg/g) compared to a control strain. To further improve the production of these compounds, the culture conditions and medium composition were optimised and the process was then scaled up to bioreactor conditions using technical and waste glycerol. The results showed that the production of phospholipids was at a similar level regardless of the type of glycerol used. Ultimately, strain PS08 was able to biosynthesise 653.7 mg/L of phospholipids, among which 352.6 mg/L was phosphatidylcholine.

Subsequent studies focused on the synthesis of the polyphenolic compound resveratrol. To this end, *Y. lipolytica* strains capable of producing it were constructed, in which the efficiency of two heterologous pathways - the tyrosine catabolism pathway

and the phenylalanine catabolism pathway - was tested. Further increases in the amount of resveratrol obtained were carried out by increasing the copy number of genes encoding key enzymes, as well as by optimising the medium composition and process conditions. The results showed that the best resveratrol producer was a strain combining both pathways of catabolism of the aforementioned amino acids, with a double copy of the genes (strain T2P2), which was able to secrete 0.104 g/L of resveratrol in flask culture. Subsequently, production of this metabolite using glycerol was carried out in a bioreactor, yielding a 4-fold higher concentration of 0.430 g/L resveratrol.

The interdisciplinary part of the study concerned the esterification of resveratrol with selected palmitic, oleic and conjugated linoleic fatty acids, and the resulting conjugates were then analysed for their anticancer and antioxidant properties against lung carcinoma (A549), colorectal adenocarcinoma (HT29), and pancreatic ductal adenocarcinoma (BxPC3) cell lines. The lipophilic versions of resveratrol molecule were aimed at extending its possible applications through increased bioavailability and stability of the new conjugates. Several parameters were investigated: cell viability and apoptosis, including the expression of major pro- and anti-apoptotic markers, as well as the expression of superoxide dismutase, a key enzyme of the body's antioxidant barrier. The results obtained allowed the selection of three esters: mono-RES-OA, tri-RES-PA and mono-RES-CLA, which, compared to the other esters, clearly showed a reduction in tumour cell viability, with no effect on normal cells. In addition, the selected esters exhibited antioxidant properties towards the normal cell line, affecting the up-regulation of the expression of major pro-antioxidant genes without affecting their expression in cancer cells, thereby reducing the defence of cancer cells against the increased oxidative stress induced by high ROS accumulation. The results showed that the resveratrol and long-chain fatty acid esters allow for an increase in their biological activity and thus their high potential for clinical application.