

Citric acid production by *Y. lipolytica* W29 from crude glycerol: strain improvement by mutagenesis

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Citric acid, an important and versatile organic acid extensively used in several industries, is originally produced by *Aspergillus niger* in submerged fermentation from molasses [1]. However, *Yarrowia lipolytica* have been studied and demonstrate a great potential as citric acid producer from several carbon sources [1–5] including crude glycerol, a low cost by-product from the biodiesel industry [6]. The simultaneous production of the isomer isocitric acid is the major problem in using this yeast in the citric acid production. Isocitric acid lower buffer capacity and chelating ability can affect citric acid crystallization, being a problem during the purification process [7]. Thus, in order obtain improved strains with reduced isocitric/citric acid ratio and/or enhanced citric acid production, *Y. lipolytica* W29 (ATCC 20460) was treated with UV-irradiation and/or ethyl methane sulfonate (EMS). Acetate-negative mutants, strains that did not grow or displayed a reduced/retarded growth, were selected for screening the citric acid production profile. Thirty seven mutant strains were selected from the acetate medium and citric acid production from crude glycerol for each strain was accessed in flask assays. From the strains tested, *Y. lipolytica* UV-75 and UV/EMS-10 stood out. The UV-75 strain presents a 4-fold lower isocitric/citric ratio comparing with the parent strain. UV/EMS-10 strain has a 1.6- and 1.9-fold higher citric acid concentration and yield, respectively than the W29 strain. Cellular growth, citric and isocitric acid production of these two strains in bioreactor fermentation were compared with *Y. lipolytica* W29. Higher concentration of citric acid was obtained by *Y. lipolytica* UV/EMS-10, which presented a 1.8-fold enhancement on citric acid concentration and 2.2-fold in citric acid yield comparing with the parent strain.

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