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EXPLOITING METABOLIC ENGINEERING FOR SECONDARY METABOLITES PRODUCTION IN *SACCHAROMYCES CEREVISIAE*

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One of the most promising applications of metabolic engineering is the biosynthesis of important lead compounds. Quinonemethide triterpene (QT) is an important minority class of secondary metabolites accumulated on Celastraceae species. Some QT have revealed potent antitumoral and antioxidant activities. In a previous work, friedelin was showed to be the key intermediate in its mevalonate pathway [1]. Based on the importance of such compounds, a metabolic engineering strategy has been developed in order to increase the production of friedelin in *Saccharomyces cerevisiae.* Along with this proposal, arise the possibility of understanding the cellular response against the metabolite disorder to generate new compounds. The coding sequence of *Kd*FRS was commercially obtained and *Mi*FRS was cloned. Both were expressed in the vector pYES2. Yeast was cultivated in synthetic medium without uracil and 2% of galactose was added to promove the induction of friedelin synthase (FS). Control was constituted by yeast strain with the vector empty. Cultures were submitted to quenching procedure, extracted with boiling ethanol solution and partitioned against $CHCl_3$. The resulting hydrophobic solutions were analyzed by GC-MS and the metabolic identification was based on the NIST library. The data was explored using unsupervised multivariate analyses including principal component analysis (PCA) and partial least square-discriminant regression analysis (PLS-DA), performed in MATLAB 8.2 using the Statistics toolbox. Optimal results regarding identity prediction were obtained with a 3-component PLS-DA model with mean center followed by pareto scaling of the units. The metabolites of mevalonate pathway from different genetic modified yeast were discriminated and classified using PLS-DA. The loading graphics indicate that the main metabolic differences are related to the concentration of steroids in the transformed yeast when compared to the control of wild yeast. Significant decrease of lanosterol and derivatives, accompanied by the production of friedelin, show the deviation of biosynthetic pathway for the production of target triterpenes when FS is encoded. In yeast with *Mi*FRS coded and designed to suppression of lanosterol synthase, was observed a significant increase of isoprenoids, mainly farnesol, geraniol and squalene, consequently favoring friedelin production, besides other triterpenes. All the results were corroborated by analysis of mass spectra and PCA. In conclusion, the metabolome analysis using statistical tools showed to be effective in interpreting and easily preliminary understand to metabolic changes in response to genetic modification.

[1] Santos, V.A.F.F.M., Santos, D.P., Castro-Gamboa, I., Zanoni, M.V.B., Furlan, M. 2010. Evaluation of antioxidant capacity and synergistic associations of quinonemethide triterpenes and phenolic substances from *Maytenus ilicifolia* (Celastraceae). Molecules. 15: 6956-6973.