Assessing the potential of micro-fermentations through screening and media optimisation of recombinant β-carotene production by the yeast Yarrowia lipolytica.

Jacobsen, Irene Hjorth; Ledesma-Amaro, Rodrigo ; Martinez Ruiz, José Luis

Publication date:
2019

Document Version
Peer reviewed version

Assessing the potential of micro-fermentations through screening and media optimisation of recombinant β-carotene production by the yeast *Yarrowia lipolytica*.

Irene Hjorth Jacobsen[1], Rodrigo Ledesma-Amaro[2], José Luis Martinez[1*]

[1]Department of Biotechnology and Biomedicine, Section of Synthetic Biology. Technical University of Denmark (DTU), Kgs. Lyngby (Denmark) [2]Faculty of Engineering, Department of Bioengineering, Imperial College London (United Kingdom

The demand for β-carotene as a natural pigment and an antioxidant has increased in previous years. All projections show that the demand will increase even more in the coming years [1], [2]. Currently β-carotene is produced either chemically or synthetically, the latter has been linked to multiple negative effects including cancer [3]–[7]. These concerns, in addition to a more critical view on synthetic colorants from the public has offset the search for natural or recombinant production platforms. Low productivities and difficulties are some of the issues associated to the few existing naturally-derived production systems [4], [6], [8].

As a result, we chose to focus on the production of recombinant β-carotene from an engineered *Yarrowia lipolytica* strain [1], and how we could optimise the production capacity as much as possible through media optimisation. In *Yarrowia*, the terpenoid pathway and lipogenesis share a common precursor, therefore we hypothesised that optimising the lipogenesis would also optimise the synthesis of β-carotene. To maximise the number of parameters in our screening we used the BioLector I micro-fermentation system, which allowed us to run 48 micro-fermentations simultaneously.

Among our findings, we identified parameters to optimise in order to target specific points in the metabolism and determined their optimum values. Through scale-up of experiments from micro-scale to benchtop laboratory-scale bioreactors we determined the scalability of the BioLector system to be accurate. We thereby proved that the BioLector I can be used in studies like ours and in studies were a fast and accurate screening method in needed.

[1] M. Larroude, E. Celinska, A. Back, S. Thomas, J. M. Nicaud, and R. Ledesma-Amaro, “A synthetic biology approach to transform Yarrowia lipolytica into a competitive biotechnological producer of β-carotene,” *Biotechnol. Bioeng.*, no. September, 2017.

[2] Grand View Research, “Beta-Carotene Market,” 2016.

[3] H. S. Black, “Pro-carcinogenic activity of β-carotene, a putative systemic photoprotectant,” *Photochem. Photobiol. Sci.*, vol. 3, no. 8, pp. 753–758, 2004.

[4] L. Bogacz-Radomska and J. Harasym, “β-Carotene—properties and production methods,” *Food Qual. Saf.*, vol. 2, no. 2, pp. 69–74, 2018.

[5] A. M. Hartman, B. K. Edwards, D. Albanes, J. Haapakoski, J. Palmgren, J. Virtamo, J. K. Huttunen, M. Rautalahti, O. P. Heinonen, and P. R. Taylor, “Effects of alpha-tocopherol and beta-carotene supplements on cancer incidence in the Alpha-Tocopherol Beta-Carotene Cancer Prevention

Study," *Am. J. Clin. Nutr.*, vol. 62, no. 6, p. 1427S–1430S, 1995.

[6] B. D. Ribeiro, D. W. Barreto, and M. A. Z. Coelho, "Technological Aspects of β-Carotene Production," *Food Bioprocess Technol.*, vol. 4, no. 5, pp. 693–701, 2011.

[7] R. A. Woutersen, A. P. M. Wolterbeek, M. J. Appel, H. van den Berg, R. A. Goldbohm, and V. J. Feron, "Safety Evaluation of Synthetic β-Carotene," *Crit. Rev. Toxicol.*, vol. 29, no. 6, pp. 515–542, 1999.

[8] A. C. Guedes, H. M. Amaro, and F. X. Malcata, "Microalgae as sources of carotenoids," *Mar. Drugs*, vol. 9, no. 4, pp. 625–644, 2011.