Expert Review

Pharmaceutical BIOPROCESSING

Diffusion Control Analysis Inspires Carotenoid Generation by a Biochemically Engineered Strain

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Abstract

Genome-scale metabolic models offer a significant advantage in systems biology because their use as metabolic flux simulation models allows for predictions of the production of industrially relevant metabolites. The biotechnological production of lycopene from Yarrowia lipolytica is a new field that has not been thoroughly investigated, particularly in terms of cultivation conditions of newly generated engineered strains. We used flux balance analysis (FBA) and Plackett-Burman design to screen chemicals for lycopene production from a metabolically engineered strain of Y. lipolytica in this study. In fed-batch cultivation, lycopene concentrations of 126 and 242 mg/L were obtained from the FBA-independent and FBA-assisted designed media, respectively. Transcriptional studies revealed up regulations of heterologous genes in FBA-designed media, implying that model predictions are accurate.

Keywords: Yarrowia lipolytica • Lycopene • Flux balance analysis • Fermentation

Introduction

Carotenoids are a type of tetraterpenoid pigmented lipid compound produced by plants as well as various fungi and bacteria. Carotenoids, which include ß-carotene, lycopene, astaxanthin, zeaxanthin, fucoxanthin, ß-cryptoxanthin, canthaxanthin, lutein, and crocetin, are responsible for a wide range of bright colours such as yellow, red, purple, and orange. Lycopene is a bioactive phytochemical in the carotenoids family that has received a lot of attention in recent years due to its commercial properties and bio functionality. Additionally, a fed-batch process for the production of lycopene from this species was developed, but the authors did not focus on improving the bioprocess route [1]. In addition, lycopene production from engineered strains of Y. lipolytica under lipid accumulation conditions has only been reported in one patent to date .Along these lines, tries should be made for building total creative, financially savvy and serious bioprocesses for lycopene creation from hereditarily designed types of Y. lipolytica. Based on the combination of computational methods and the knockout or overexpression of particular genes, one of the most effective methods for producing high titers of carotenoids in engineered strains is [2].

Lycopene is essential for the human body because it can only be ingested in multiple forms, such as food or drugs, and it is only synthesized by plants like tomato, watermelon, guava, papaya, apricots, pink grapefruit, and red oranges, as well as by carotenogenic microorganisms. Due to its therapeutic, preventative, and nutraceutical properties, lycopene is beneficial to health. Its implications in carcinogenic cell apoptosis and cell cycle arrest of various human cancers are being demonstrated by increasing scientific evidence. Additionally, it has been demonstrated that lycopene has cardio protective, antioxidant, and anti-inflammatory properties, making it a potent cancer chemo preventive agent. Given the foregoing, the mass production of lycopene is absolutely necessary. However, issues with food competition, the environment, high production costs, lengthy processing times, and low yields hinder the current production of lycopene from plant sources. Additionally, carotenogenic microorganism-based microbial fermentation

is rare and does not yield significant lycopene concentrations. Numerous efforts have been made to engineer lycopene-producing hosts that are not carotenogenic; however, the main obstacle to producing a lot of lycopene is still the fermentation process [3]. As a promising fermentation platform, Yarrowia lipolytica, which is GRAS (Generally Recognized as Safe), has been established.

Discussion

The Plackett-Burman design and other statistical design techniques are frequently used to improve fermentation media. For studying N-1 variables using N runs, where N is a multiple of 4, the Plackett-Burman designs are typically resolution III, highly efficient two level fractional factorial screening designs. They quickly identify the factors that have a significant impact on the response by using the smallest number of runs. However, the initial set of chemical compounds is difficult to select because they are typically chosen at random. In addition, despite the fact that GSMN is able to predict the nutritional requirements of a particular biological system, it is challenging to ascertain the precise quantity of a single predicted compound that should be added due to the fact that in silico data rarely coincide with the metabolic behavior that occurs in vivo. However, an effective culture medium for the production of valuable objective products can be created by combining GSMN (FBA) results with statistical techniques like Plackett-Burman design [4].

As a result, in this paper, we run FBA using COBRA Toolbox 2.0 to anticipate environmental conditions that contribute to FPP overproduction and may well redirect carbon flux toward lycopene biosynthesis in an engineered strain of Y. lipolytica with only crtE, crtB, and crtI genes. We combined modelling data with the Plackett-Burman factorial design of experiments to optimize cultivation media because model predictions do not accurately reflect the situation in vivo. In addition, through fermentation scale-up experiments, we compared the production of lycopene in modelling-suggested media to that in other media based on commonly reported compounds [5].

A comprehensive method for engineering Y. lipolytica to produce lycopene has recently been developed. However, other than the culture medium and genetic manipulation procedure described in, no in-depth examination of cultural conditions was provided. Additionally, a fed-batch process for the production of lycopene from this species was developed, but the authors did not focus on improving the bioprocess route. In addition, lycopene production from engineered strains of Y. lipolytica under lipid accumulation conditions has only been reported in one patent to date. Along these lines, tries should be made for building total creative, financially savvy and serious bioprocesses for lycopene creation from hereditarily designed types of Y. lipolytica. Based on the combination of computational methods and the knockout or overexpression of particular genes, one of the most effective methods for producing high titers of carotenoids in engineered strains is. In addition, successful genome-scale metabolic network (GSMN) reconstruction has been facilitated by whole-genome sequencing, high-throughput omics, in-depth biochemical and enzymatic documentation on microbial metabolism, and both of these methods [6].

Results

The exchange reactions involving amino acids like l-isoleucine, l-leucine, l-valine, l-asparagine, l-histidine, l-methionine, l-tryptophan, guanine, and l-lysine were the most common type.O2, thiamine diphosphate, succinate, phosphate, ammonia, 4-aminobutanoate, ergosterol, ethanolamine, d-fructose, urea, d-glucose, hypoxanthine, and (R)-pantothenate were the additional exchange reactions [7]. Depending on the type of carbon source chosen, there was a wide range in the number of negative exchange reactions and fluxes. The only "non-zero" values appear to be those corresponding to O2 exchange, d-fructose exchange, and d-glucose exchange, despite the fact that FBA only provides one random solution rather than considering all of the possibilities (as Flux Variability Analysis would, for instance).All other values between 8.05e-35 and 1.46e-13 could be regarded as zeros due to the LP solver's most likely rounding errors. We relaxed the exchange reactions of model yli v1.7, however, in order to predict the potential benefit of supplementing the minimal media with the aforementioned amino acids or a number of cofactors in terms of FPP production based on FBA metabolic flux distributions [8] .We first tried out the effects of 18 amino acids—only 18 amino acids can be used in model yli v1.7—on FPP production at a specific growth rate (90 percent of the maximum growth rate). We found that each of the 18 amino acids helped FPP production in different ways.However, due

to the guarantee of a C/N ratio greater than 10, only eight amino acids—l-isoleucine, l-leucine, l-valine, l-asparagine, l-histidine, l-methionine, l-lysine, and l-tryptophan—were identified as amino acids that could be added to the minimal medium.We also tested the effects of 10 other factors on FPP production at a specific growth rate. We discovered that some of these factors had a significant impact on FPP production. In addition, the minimal medium was supplemented with all ten factors to examine their effects on FPP production [9, 10].

Conclusion

The FBA and Plackett–Burman screening investigational design made it possible to introduce significant variables that affected Y. lipolytica's lycopene production .Improved lycopene production from Y. For the transfer of air into the bioreactor, sterile air filters with pores of 0.2 m were utilized .Using a pO2 electrode, the concentration of DO in the culture broth was measured. Silicone was occasionally added as an antifoam specialist. The fed-batch process consisted of supplementing a feeding solution with 200 g/L of glucose and 200 g/L of fructose for 24 hours. Feedings of 50 mL were given every 24 hours, and the pH was kept at 5.5 at first and 3 at 48 hours after the colour appeared .lipolytica as a fermentation platform and increased industrial production of terpenoids may result from the current study's findings. On the journal website, additional information regarding Pareto charts of the Plackett-Burman design and the Plackett-Burman design regarding the expression levels of terpenoids' backbone genes is available. The coloured mixed supernatants were then transferred into a new tube after the mixture was centrifuged for ten minutes at 12,000 rpm. The extraction step was rehashed until all noticeable shades in the remaining cell pellet and supernatant were extricated. High-performance liquid chromatography (HPLC) analysis was applied to the pooled coloured supernatants for the purpose of determining the amount of lycopene in the mixture. To prevent photo degradation, isomerization, and structural changes in the carotenoids, all operations were carried out on ice in dim lighting. The agitation rate was set to automatically respond to the dissolved oxygen (DO) concentration to keep it at 50%, and the aeration rate was set to 1vvm (volume air per volume per minute).

Acknowledgement

None

Conflict of Interest

None

References

1. Brittberg M, Lindahl A, Nilsson A, *et al.* Treatment of Deep Cartilage Defects in the Knee with Autologous Chondrocyte Transplantation. *N. Engl. J. Med.* 331, 889–895(1994).
2. Wood F, Martin L, Lewis D *et al.* A Prospective Randomised Clinical Pilot Study to Compare the Effectiveness of Biobrane Synthetic Wound Dressing, with or without Autologous Cell Suspension, to the Local Standard Treatment Regimen in Paediatric Scald Injuries. *Burns.* 38, 830–839(2012).
3. Mancardi G, Saccardi R. Autologous Haematopoietic Stem-Cell Transplantation in Multiple Sclerosis. *Lancet. Neurol.* 7, 626–636(2008).
4. Braendstrup P, Levine BL, Ruella M. The Long Road to the First FDA-Approved Gene Therapy: Chimeric Antigen Receptor T Cells Targeting CD19. *Cytotherapy.* 22, 57–69(2020).
5. Moutsatsou P, Ochs J, Schmitt RH. Automation in Cell and Gene Therapy Manufacturing: From Past to Future. *Biotechnol. Lett.* 41, 1245–1253 (2019).
6. Lam C, Meinert E, Yang A *et al.* Comparison between Centralized and Decentralized Supply Chains of Autologous Chimeric Antigen Receptor T-Cell Therapies: A UK Case Study Based on Discrete Event Simulation. *Cytotherapy.* 23, 433–451(2021).
7. Decker WK, da Silva RF, Sanabria MH *et al.* Cancer Immunotherapy: Historical Perspective of a Clinical Revolution and Emerging Preclinical Animal Models. *Front. Immunol.* 8, 56-65(2019).
8. Dunn GP, Old LJ, Schreiber RD. The Three Es of Cancer Immunoediting. *Annu. Rev. Immunol.* 22, 329–360(2014).
9. Clemente CG, Mihm MC, Bufalino R. Prognostic Value of Tumor Infiltrating Lymphocytes in the Vertical Growth Phase of Primary Cutaneous Melanoma. *Cancer.*77, 1303–1310(1996)
10. Borst J, Ahrends T, Bąbała N *et al.* CD4+ T Cell Help in Cancer Immunology and Immunotherapy. *Nat. Rev. Immunol.* 18, 635–647(2018).