Optimising the biosynthesis of oxygenated and acetylated Taxol precursors in Saccharomyces cerevisiae using advanced bioprocessing strategies

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Abstract

Taxadien-5α-hydroxylase and taxadien-5α-ol O-acetyltransferase catalyse the oxidation of taxadiene to taxadien-5α-ol and subsequent acetylation to taxadien-5α-yl-acetate in the biosynthesis of the blockbuster anti-cancer drug, paclitaxel (Taxol). Despite decades of research, the promiscuous and multispecific CYP725A4 enzyme remains a major bottleneck in microbial biosynthetic pathway development. In this study, an interdisciplinary approach was applied for the construction and optimisation of the early pathway in Saccharomyces cerevisiae, across a range of bioreactor scales. High-throughput microscale optimisation enhanced total oxygenated taxane titre to 39.0±5.7 mg/L and total taxane product titres were comparable at micro and mini-bioreactor scale at 95.4±18.0 and 98.9 mg/L, respectively. The introduction of pH control successfully mitigated a reduction of oxygenated taxane production, enhancing the potential taxadien-5α-ol isomer titre to 19.2 mg/L, comparable to the 23.8±3.7 mg/L achieved at microscale. A combination of bioprocess optimisation and increased GC-MS resolution at 1L bioreactor scale facilitated taxadien-5α-yl-acetate detection with a final titre of 3.7 mg/L. Total oxygenated taxane titres were improved 2.7-fold at this scale to 78 mg/L, the highest reported titre in yeast. Critical parameters affecting the productivity of the engineered strain were identified across a range of scales, providing a foundation for the development of robust integrated bioprocess control systems.

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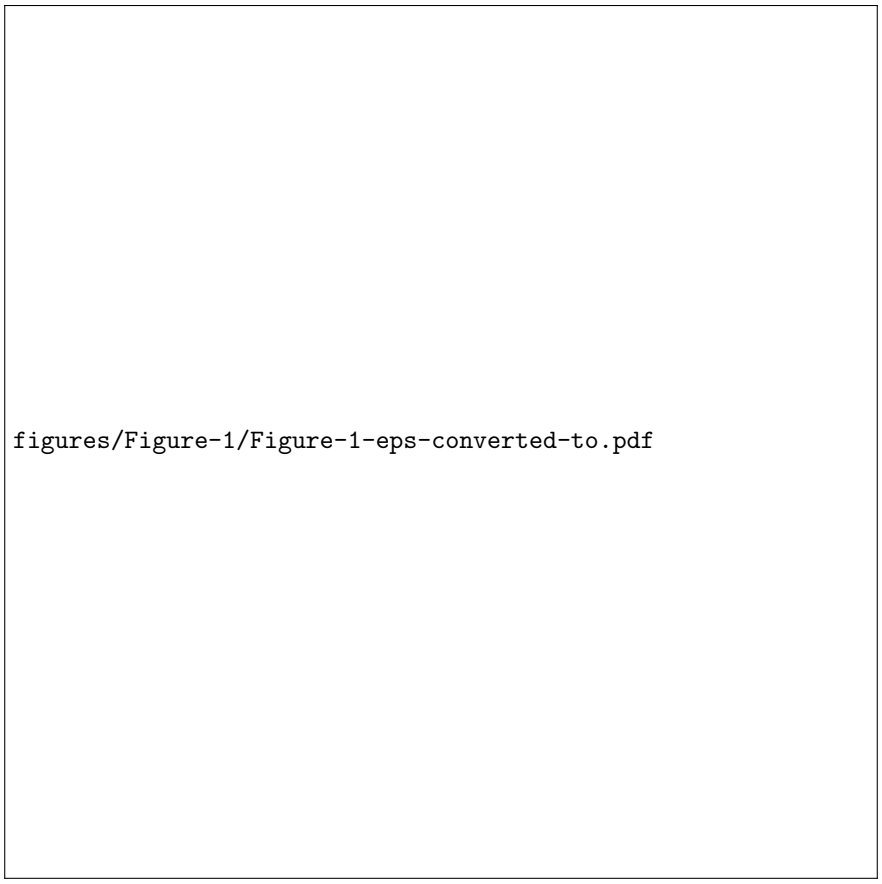

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Table 1.docx available at https://authorea.com/users/331623/articles/458257-optimising-the-biosynthesis-of-oxygenated-and-acetylated-taxol-precursors-in-saccharomyces-cerevisiae-using-advanced-bioprocessing-strategies