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Production of indigo in the cyanobacterium Synechocystis sp. 6803 by expression of a flavin-containing monooxygenase

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PURPOSE OF THE ABSTRACT

Production of indigo in the cyanobacterium Synechocystis sp. 6803 by expression of a flavin-containing monooxygenase

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Indigo is one of the most produced dyes in the world; except for being famous for the colouring of denim, it has a wide variety of other uses. It is currently produced by chemical processes putting a heavy burden on the environment [1]. To answer the demand for clean synthesis routes, biotechnological processes are being developed based on the employment of enzymes. Flavin-dependent monooxygenases (FMOs), naphthalene dioxygenases or cytochrome P450 are explored as biocatalysts in different configurations, i.e. as isolated (engineered) recombinant enzymes or in whole-cell biotransformations using recombinant expression in E. coli [2]. These oxidative enzymes catalyse the oxidation of indole to indoxyl, resulting in indigo formation. In turn, the substrate indole can be produced, together with pyruvate and ammonia, from L-tryptophan by employing tryptophanase (TRP). To our knowledge, the highest yield reported in the literature for such two-step system is 1,7 g of indigo per liter of culture grown for 3,5 days in a medium containing 2 g/L of l-tryptophan [2].
The use of isolated enzymes to drive redox reactions is hampered by their need of highly expensive redox cofactors such as NADPH. Cofactor regeneration is one of the main bottlenecks for the application of such biocatalysts at an industrial scale [3]. Recently, the possibility to use the photosynthetic apparatus of cyanobacteria for NADPH (re)generation has emerged. Among all cyanobacteria, Synechocystis sp. PCC6803 is progressively attracting interest as cell factory for the production of commodity chemicals, fuels and value-added products by essentially consuming carbon dioxide and water using light as energy source. Synechocystis strains expressing different enzymes have already been successfully tested in biotransformations in whole-cell configurations [4].
We have produced a Synechocystis strain constitutively expressing the FMO from Methylophaga aminisulfidi¬vorans (mFMO). Grown in batch and fed with 0.12 g/L indole as substrate, this strain produced 0.10 g/L of indigo. Noteworthy, indigo was found to be present in the supernatant in the form of small, dark blue agglomerates attached to the flasks. As stated above, our interest is to carry out the two-step cascade starting from L-tryptophan and to enhance the yield of indigo. We have therefore produced a second Synechocystis strain expressing both enzymes, TRP and mFMO, either encoded on an operon or expressed as a bifunctional fusion enzyme [2]. We will report on the results and the progress of this work.

FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

Whole-cell biocatalysis | Indigo dye | Synthetic biology | Synechocystis

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