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Optimized synthesis of plant lignans via chromosomal integration of a multi-enzyme cascade in E. coli

AUTHORS
Ronja KNÖFEL / HEINRICH-HEINE-UNIVERSITÄT, UNIVERSITÄTSSTRASSE 1, DÜSSELDORF
Vlada B. URLACHER / HEINRICH-HEINE-UNIVERSITÄT, UNIVERSITÄTSSTRASSE 1, DÜSSELDORF
U. Joost LÜLF / HEINRICH-HEINE-UNIVERSITÄT, UNIVERSITÄTSSTRASSE 1, DÜSSELDORF
Philipp A. BECHTOLD / HEINRICH-HEINE-UNIVERSITÄT, UNIVERSITÄTSSTRASSE 1, DÜSSELDORF

PURPOSE OF THE ABSTRACT
The lignans (-)-deoxypodophyllotoxin and (-)-podophyllotoxin are precursors in the synthesis of the chemotherapeutic etoposide [1]. To overcome their limited availability in the natural source - the endangered plant Sinopodophyllum hexandrum – alternative routes have been suggested, including chemo-enzymatic synthesis [2,3] and the heterologous production in tobacco leaves [4]. The genes coding for the enzymes involved in the biosynthetic pathway from (+)-pinoresinol to (-)-deoxypodophyllotoxin in S. hexandrum has been deciphered [5]. In our previous studies, we introduced the heterologous biosynthesis of lignans in Escherichia coli [1, 6]. The cascade included eight genes from four plants which were cloned in several plasmids. To ensure the redox cofactor availability, the cascade was divided between two E. coli cells [1].

Generally, the use of multiple plasmids suffers from plasmid-instability, reduced cell growth, higher costs due to the need for e.g. antibiotics and high cell-to-cell variability. In this study, we integrated the genes encoding the four-step cascade from (+)-pinoresinol to (-)-pluviatolide, an important cross-road compound of lignans and precursor of (-)-deoxypodophyllotoxin synthesis, into the E. coli chromosome. Pluviatolide synthesis in resting and growing recombinant cells was analyzed for both episomal and chromosomal expression systems. Energy and carbon sources were compared and the effect of cell permeabilization on product titers investigated. Product analysis was done by LC/MS.

Our results revealed that the biotransformation with resting cells yielded similar quantities of the product (-)-pluviatolide, independently on the type of gene expression. In contrast, growing cells with chromosomally integrated foreign genes performed better which led to doubled pluviatolide titers. Generally, the addition of glucose or glycerol had a positive impact on biotransformation. While glycerol improved the productivity of non-permeabilized resting cells, permeabilized cells performed best after glucose supplementation. In both cases up to 99% (+)-pinoresinol were converted in (-)-pluviatolide. In growing cells, the biotransformation was limited by cell death after 24 hours, but the addition of glucose as carbon and energy source led to further cell growth and yielded 99% (-)-pluviatolide.

FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

Plant lignans | E. coli | Multi-enzyme cascades | Chromosomal integration

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