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BOOK OF ABSTRACTS

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I10. Industrial and Food Microbiology and Biotechnology

P387. Combinatorial biosynthesis of plant natural products in microorganisms

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Plant natural products (PNPs) are among the most significant compounds used in pharmaceutical and cosmetic industries. However, most PNPs accumulate at low quantities in plants and are difficult and expensive to isolate. Additionally, a high investment of land, water and time is required and pests and extreme weather cause insecurity in the supply chain. Moreover, their chemical synthesis is challenging due to their stereochemical complexity. Therefore, in the last decades, PNPs pathways have been engineered in microbial hosts using combinatorial biosynthesis. In this approach, genes from different species are assembled to construct complex biosynthetic pathways. Curcuminoids are PNPs whose biosynthetic pathways have been extensively explored in the last years due to their applications. They have been used in traditional food, cosmetic and medicine for centuries. Their therapeutic properties include anti-cancer, anti-inflammatory, anti-oxidant, anti-Alzheimer's, and anti- HIV, among others. Curcumin, the most promising curcuminoid, has a projected market size of USD
130 million by 2025. Herein, we propose an optimized artificial biosynthetic pathway to produce curcuminoids. This pathway involves 6 enzymes and produces ferulic acid as an intermediate using caffeic acid O-methyltransferase. Starting from tyrosine, 1325 µM of ferulic acid were obtained, comprising the first part of the pathway. Then, the second part of the pathway was also optimized. From ferulic acid we obtained the highest concentration of curcumin reported (1212.7 µM) so far, corresponding to a 26% increase [1]. Subsequently, curcumin was produced from tyrosine (the whole pathway) using a mono-culture. Production increased comparing to a previously reported pathway that used a caffeoyl-CoA O-methyltransferase [2]. Additionally, a co-culture strategy was evaluated to further improve the production by reducing cells metabolic burden. We used one *E. coli* strain able to convert tyrosine to hydroxycinnamic acids and another able to convert them to curcuminoids. Using CRISPR-Cas9 method we disrupted *lacZ* gene in one of the strains which allowed to follow co-culture population composition using the blue-white screening method. This co-culture strategy increased 8.8 times the curcuminoids production (126 µM) as compared to the mono-culture production. These results comprise a significant step towards the large-scale production of these valuable compounds.