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Volatile Compounds' Profiling and Engineering of *Ashbya Gossypii* Strains for *De Novo* Biosynthesis of Lactones.

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Lactones are highly valuable cyclic esters of hydroxy fatty acids that find application as pure fragrance compounds or as building blocks for fine/specialty chemicals synthesis1. While chemical synthesis often leads to undesired racemic mixtures, the microbial production of lactones offers the possibility to obtain optically pure lactones from natural sources. Therefore, the biotransformation of hydroxy fatty acids to lactones has been explored as the preferred path towards the establishment of industrial-scale production processes. However, the production of a specific lactone depends on the availability of the corresponding hydroxy fatty acid1, which often has economic value and industrial applicability equivalent to that of lactones. Accordingly, the identification of microorganisms with the rare natural ability for de novo biosynthesis of lactones constitutes a major challenge for this field.

Ashbya gossypii is a filamentous fungus currently used for the industrial production of riboflavin (vitamin B2) that has a very rich and heterogeneous secondary metabolism2. With this in mind, we characterized by GC/MS the metabolic profile of the volatile compounds (VOCs) produced by 11 A. gossypii strains in standard sugar-based medium and identified up to 7 chemically different lactones produced by each strain, some of which at concentrations above their odour perception threshold. To further improve the A. gossypii de novo biosynthesis of lactones from glucose, we developed metabolic engineering strategies focused on oleic acid3,4 as the central precursor. A. gossypii was thus engineered to: i) accumulate more oleic acid by blocking the fatty acid biosynthesis pathway at the C18 level and by redirecting the metabolic flux towards linoleic acid formation through the deletion of AgELO624 and overexpression of AgDES589, respectively; ii) stop the degradation of fatty acids at the C10 level and consequently channelling the production of lactones towards γ-decalactone through the substitution of the AgPOX1, which codifies for an unspecific oxidase from the β-oxidation pathway, by a codon-optimized POX2 gene from Yarrowia lipolytica codifying a specific long chain oxidase with activity towards C18–C11 fatty acids. Overall, combinatorial engineering allowed improving the production of total lactones by 7-fold (7584 µg/gCDW) and fine-tuning the biosynthesis of γ-decalactone, which represented more than 99% of the total lactones produced. These results thus demonstrate the potential of A. gossypii as an early platform for de novo biosynthesis of lactones from glucose. Moreover, they provide compelling proof-of-concept data for the production of lactones from carbohydrates, which are abundant in industrial wastes and renewable raw materials.

1. Kourist and Hilterhaus, in:Microbiology Monographs,26.
2. Aguiar et al., Biotechnol Adv 2015;33:1774–86.
3. Sánchez et al., BMC Genomics 2013;23:343.
4. Sánchez-Sevilla et al., BMC Genomics 2014;15:218.

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