Transgenic Anthriscus sylvestris for the biosynthesis of lignans

Metabolic engineering strategies for the optimization of medicinal and aromatic plants

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SCIENTIFIC HIGHLIGHTS

Plants and microorganisms are important sources for gene and drug discovery. Most isolated natural products with drug potential are structurally too complex, their isolation is too expensive or they can only be obtained in very low quantities. To overcome these problems, in combinatorial biosynthesis plant pathways are copied in microbial organisms being capable as biological factories to produce natural compounds of interest.

Etoposide and tenoposide are two major anticancer drugs in clinics today. Both are semi-synthesized based on podophyllotoxin (PTOX) what is isolated from nearly extincted plants from the Himalaya region. *Anthriscus sylvestris* is an alternative common weed in Northern Europe producing lignans. By cloning the human cytochrome P450 in *A. sylvestris*, we modified the plant genetically to produce PTOX. Plant secondary lignan metabolism is now under investigation and breeding studies are carried out with partners.

Fig. 1 *Anthriscus sylvestris*, Wiesenkerbel

Additional isolation of endophytes showed a new approach for PTOX production. Endophytes are in plants symbiontic living fungi or bacteria producing secondary natural products like lignans. They are of main interest to understand the genetic blueprint and to learn about gene clustering. By metabolomic studies metabolite accumulation is documented and optimal growth conditions is determined.

Highlight: Collection of *Anthriscus sylvestris* seeds from 15 locations in North Europe, cultivation of plants under uniform indoor and recorded outdoor conditions.

Highlight: Identification of a new biosynthetic side pathway to 6-Hydroxy-lignans. In an ongoing project we identified by LC-NMR-MS most of the biosynthetic precursors.

Highlight: Cloning of cytochrome P450 3A4 to *Anthriscus sylvestris* and regeneration of the plant. By *Agrobacterium tumefaciens* transformation the above mentioned gene was cloned *to E. coli*, *S. cerevisieae* and *A. sylvestris*.

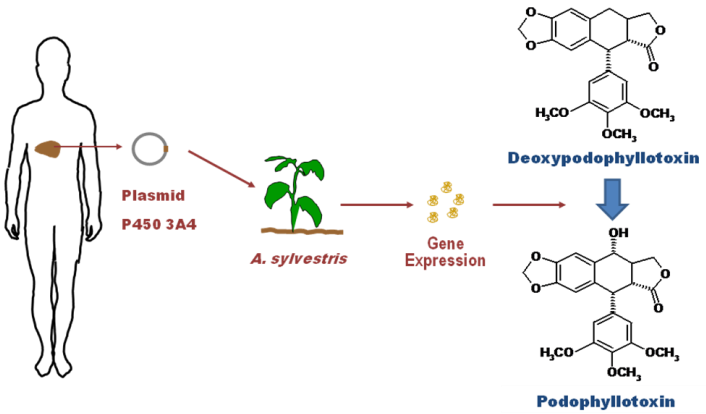

Fig. 2 Principle of combinatorial biosynthesis of PTOX. Human liver cytochrome P4503 A4 is cloned in *A. sylvestris* to allow last catalytic conversion from deoxypodophyllotoxin to PTOX.

Highlight: Mechanistic studies to explain the stereoselective mode of hydroxylation at C-7 of deoxypodophyllotoxin to PTOX by Cyp450 3A4.

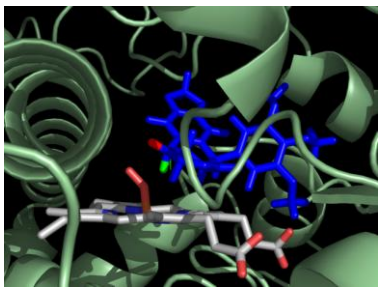

Fig 3 Stereoselectivity of the solutions towards β-hydrogen atom (green color) at C-7 position of DOP compared to α-hydrogen atom (red color)

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