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# CONSTRUCTION OF A RECOMBINANT BIOCATALYST FOR THE PRODUCTION OF PHENYLACETIC ACIDS AND PHENYLETHANOLS FROM STYRENES

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## Background

Numerous soil bacteria have been reported to be able to metabolize styrene via the pathway of side-chain oxygenation. This pathway comprises a styrene monooxygenase (**SMO**), which oxidizes styrene to styrene oxide, a styrene oxide isomerase (**SOI**), which converts styrene oxide into phenylacetaldehyde, and a phenylacetaldehyde dehydrogenase (**PAD**). The latter enzyme enables the oxidation of the aldehyde to the central metabolite phenylacetic acid [1].

In this study the construction of a recombinant biocatalyst under consideration of suitable SMOs, SOIs and PADs was intended because this pathway is of potential relevance for the biotechnological production of phenylacetic acids and similar compounds.

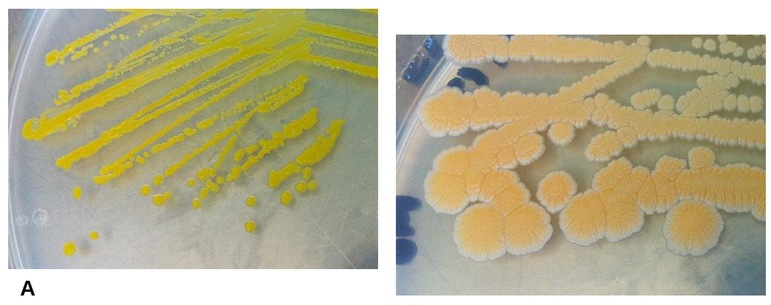


**Fig. 1: Examples of styrene-degrading soil bacteria: A *Sphingopyxis fribergensis* Kp5.2, B *Rhodococcus* sp. 5.3_2_1**

## Construction of recombinant biocatalysts harboring genes of the styrene-degrading pathway

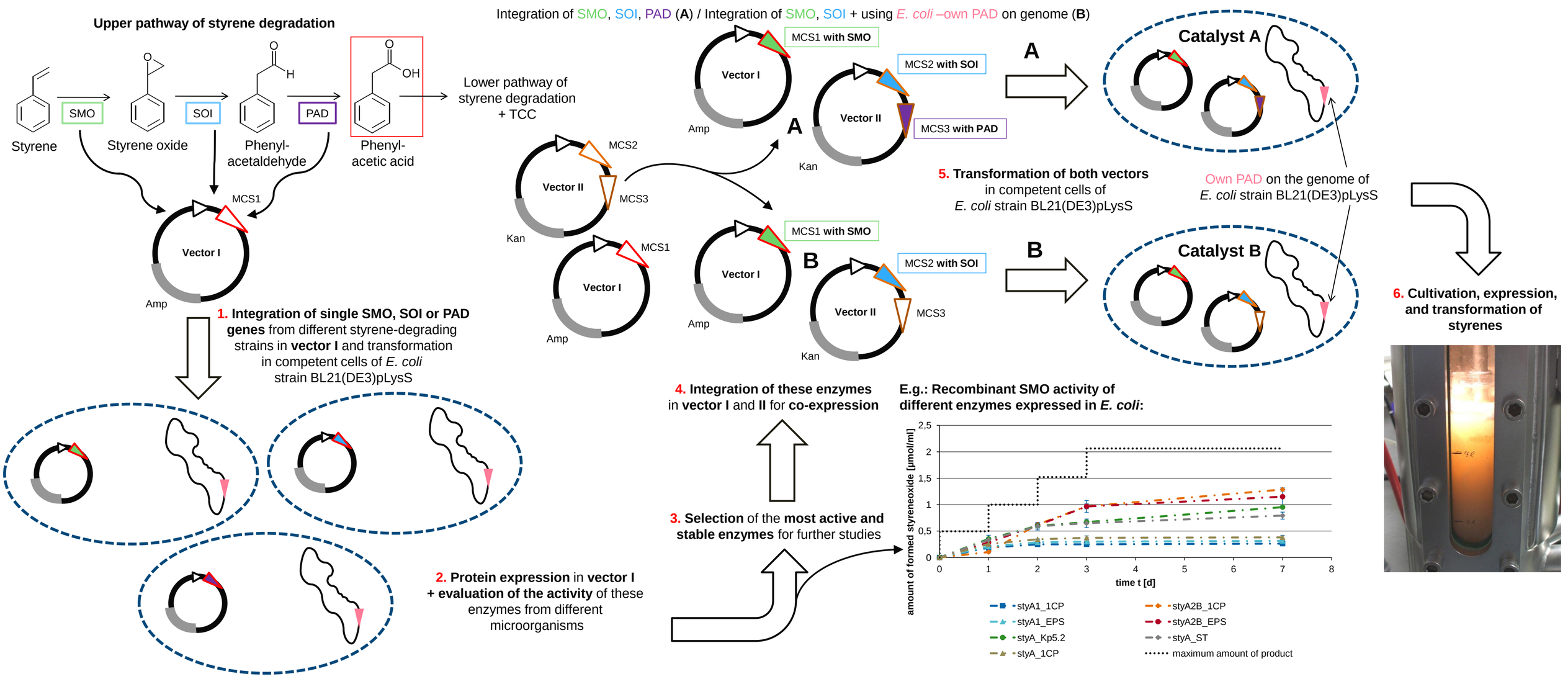


**Fig. 2: Strategy for the selection of the most active representatives of styrene monooxygenases (SMOs), styrene oxide isomerase (SOIs) and phenylacetaldehyde dehydrogenase (PADs) and their transformation from wild-type cells into *Escherichia coli* BL21(DE3)pLysS for co-expression.**

## First results: application of the biocatalysts for the transformation of styrenes

Recombinant co-expression of SMO, SOI and PAD in **biocatalyst A**:

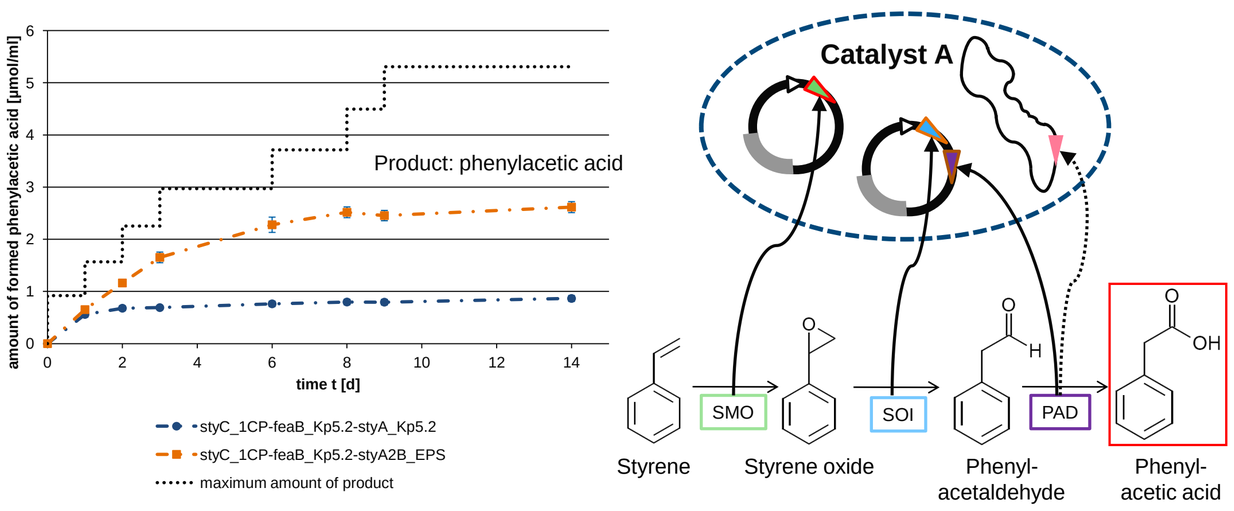


**Fig. 3: Transformation of styrene with biocatalyst A → led to the accumulation of phenylacetic acid**

Recombinant co-expression of SMO and SOI in **biocatalyst B**:

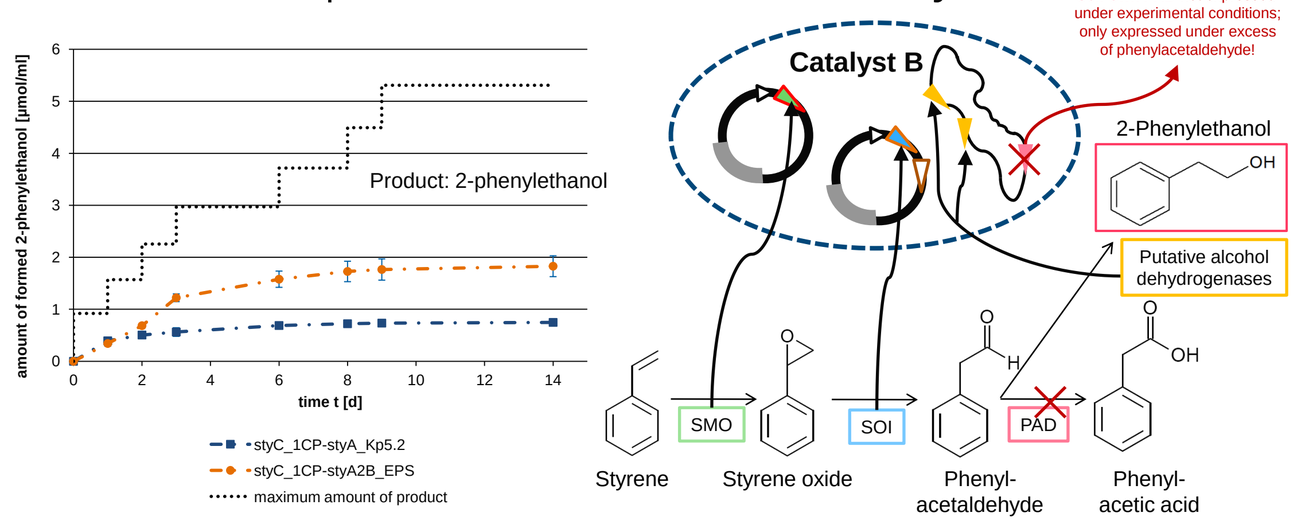


**Fig. 4: Transformation of styrene with biocatalyst B → led to the accumulation of 2-phenylethanol**

## Conclusion

Two biocatalysts were constructed harboring genes of the styrene-degradation. Therefore, the corresponding enzymes were expressed recombinantly and investigated with respect to their activity. The most promising enzymes were selected and used to construct an enzyme cascade in *Escherichia coli* BL21 (DE2)pLysS. **Biocatalyst A** contains a recombinant **SMO**, **SOI** and **PAD** and allows the synthesis of **phenylacetic acids** from styrenes. **Catalyst B** harbors only a recombinant **SMO** and **SOI**, but a **native PAD**. Remarkably, this **native PAD** is not expressed while putative **alcohol dehydrogenases** allow the synthesis of **2-phenylethanols** instead of phenylacetic acids. Both catalysts are actually objectives for further optimization with respect to cultivation, biomass production and styrene transformation.

[1] O'Leary, N. D., K. E. O´Connor, A. D. W. Dobson. 2002. FEMS Microbiol Rev **26**:403-417.

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