**INTRODUCTION**

Carotenoids and derivatives thereof (e.g. retinoids) are valuable compounds due to their numerous applications in the food and cosmetic industry, but also due to their beneficial effects on human health. Today, efficient synthesis processes are only available for a few carotenoids [1]. Thus, novel approaches to get access to less abundant or complex carotenoids are required.

The current study aimed to develop a biosynthetic route to retinoic acid, which is used for the treatment of acne and acute promyelocytic leukemia. The cleavage of β-carotene by a β-carotene 15,15'-monooxygenase (BCMO) is the first step in the synthesis of various retinoids resulting in the formation of retinal. Retinal is then converted to retinoic acid by the action of retinal dehydrogenase (RaiDH). Thus, the β-carotene biosynthesis pathway was implemented into *P. pastoris* and extended with the corresponding enzymes to produce retinoic acid (see Figure 1).

**β-CAROTENE PRODUCING PLATFORM STRAIN**

The first step was to set up a *P. pastoris* strain efficiently producing β-carotene as a platform strain that can also be exploited for the production of other interesting carotenoid derivatives.

To implement the four genes of the β-carotene pathway from *P. ananatis*, a polycistronic expression construct based on self-processing 2A sequences was designed (see Figure 2) [2]. The resulting recombinant *Pichia* strains produced up to 3.5 mg β-carotene/gCDW.

**RETINOIC ACID PRODUCTION**

Genes coding for BCMO and RaiDH from various organisms were tested for functional expression in *P. pastoris* in a first step. BCMO from chicken and RaiDH from rat showed the highest in vivo activities. Consequently, a polycistronic expression construct was designed and implemented in the β-carotene producing platform strain. Thus, retinoic acid was produced at levels of 0.18 mg/gCDW in small scale cultivations (50 mL) of the corresponding *Pichia* strains (see Figure 3).

After this first proof of concept, further pathway optimization will be required to increase the retinoic acid titers. β-Carotene was still present in the analysed cell extracts, indicating that the BCMO catalysed step did not result in full conversion. Increasing the BCMO expression levels might alleviate this problem. However, no retinal was observed indicating that the RaiDH catalysed step was efficient.

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**Figure 1:** A biosynthetic route to retinoic acid. The implementation of seven heterologous enzymes in *P. pastoris* is necessary to build a pathway in which farnesyl diphosphate is converted to retinoic acid via β-carotene.

**Figure 2:** Implementation of the β-carotene pathway in *P. pastoris*. A) Schematic representation of the polycistronic pathway construct. B) Expression of the polycistronic pathway construct resulted in homogenously orange coloured cells.

**Figure 3:** Evaluation of the in vivo production of retinoic acid. (A) HPLC analysis (λ= 345 nm) of cell extract obtained from a *P. pastoris* strain expressing a total of 7 pathway enzymes. (B) All-trans retinoic acid (red) and 9-cis retinoic acid (blue) standards.

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**References**


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