Exploiting fungal cell factories for pigment production

The growing concern over harmful effects of synthetic colorants has led to an increased interest in natural coloring alternatives. Currently, natural colorants are extracted from fruits or roots and their production is thus highly dependent on the supply of the extraction source, which can fluctuate heavily between seasons. To overcome limitations in supply, quantity and quality, alternative routes to securing a stable, sustainable production of natural colorants for food applications are required. Here, the use of fungal cell factories for pigment production is at the focal point of interest.

Monascus Pigments are fungal pigments derived from Monascus species with yellow to red color hues. They usually occur in a plethora of different pigment compounds and their exact composition is thus hard to determine or to control. Monascus Pigments have been used in the Asian food industry for centuries, however since their production is associated with the mycotoxin citrinin, Monascus Pigments are not approved for human consumption in neither Europe nor the USA. Different Monascus Pigment producing species - other than Monascus - have been described in the literature so far, one of them being Talaromyces atroroseus. T. atroroseus has recently been shown to excrete large amounts of Monascus Pigments, without production of mycotoxins. Some of the pigments are known, such as PP-O, PP-V, PP-Y, but the majority of the produced pigments have not been previously structurally characterized.

The major aim of this thesis is to propose new microbial cell factories for reliable pigment production. To do so, the potential of the red pigment producer T. atroroseus was thoroughly investigated. Firstly, different media compositions were screened and it was shown that red pigment production of T. atroroseus was enhanced using KNO3 as the nitrogen source and sucrose as the carbon source. A suitable cultivation medium was proposed and T. atroroseus was shown to be reproducibly cultivable in stirred tank reactors, which is a crucial requirement for industrial fungal cell factories. In a second step, process parameters were investigated and it was demonstrated that pigment production T. atroroseus was heavily influenced by pH and temperature. Cellular performance in terms of fungal growth was enhanced at pH 3, but pigment production was greatly increased at a pH of 4.5. It was shown that there is a clear trade-off between propagation of T. atroroseus and pigment production since no tested value of pH supported both equally well. In order to promote growth and pigment production, a cultivation method comprising a pH switch from pH 3 to pH 4.5 was developed. By employing this method, pigment production by T. atroroseus could be increased by 35%.

Furthermore, in collaboration with DTU Natural Product Chemistry different chemical analysis tools were used to investigate the pigment production portfolio of T. atroroseus. Here, we confirmed that the strain IBT 11181 produces the known Monascus Pigment PP-V and for the first time, both the cis- and trans- form of PP-O were described. Additionally, as a result of this collaboration, a new series of Monascus Pigment derivatives of the known azaphilone pigment PP-O has been characterized. These have been named atrorosins and have different amino acids incorporated into the azaphilone core.

Finally, a fermentation process tailoring pigment production in T. atroroseus was designed to selectively produce only one atrorosin at a time. By doing so, pigment purification was greatly simplified. T. atroroseus was cultivated with single amino acids as nitrogen source. The presence of an amino acid in excess promoted production of only the atrorosin derivative of that particular amino acid. All amino acids promoted growth of T. atroroseus but the pigment yield greatly varied and was best with histidine, serine and glutamate. To improve the set-up, a two-step fermentation process for production of the serine-derivative was developed. Here, in a first step nitrate limiting conditions promoted the production of cis- and trans-PP-O. In a second step, the amino acid serine was added to the cultivation medium, converting both PP-O isomers into cis- - atrorosin-S. This process design yielded essentially pure cis- atrorosin-S and final pigment titers of 0.9 g/L were reached. To the best of our knowledge, for the first time, production yields of Monascus Pigments could be given in g/L and not in Absorbance Units.

Altogether, the work presented here, has established a stable, quantifiable and high yielding cultivation process for T. atroroseus and sets therewith the cornerstone in implementing pigment production in fungal cell factories.

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