Projects of the second transnational Joint Call

MESIAB

Multi-enzyme systems involved in astin biosynthesis and their use in heterologous astin production

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Aster tataricus (left); A. tataricus (right): dried roots of A. tataricus from which astins can be extracted

Extracts from the dried roots of the plant Aster tataricus are used in traditional Chinese medicine for their various health promoting effects and antibacterial activities.

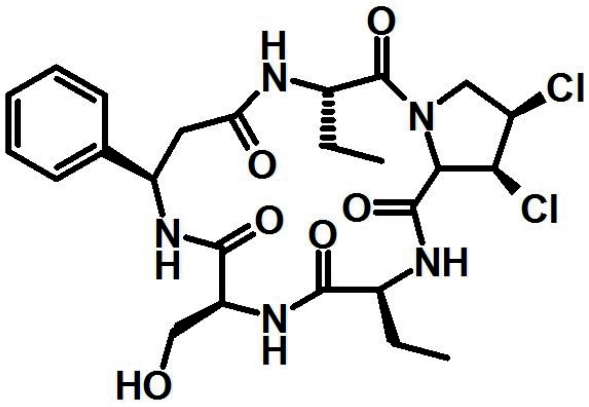
The extracts also contain astins, which are cyclic pentapeptides with an anti-tumor activity. Since the amount of astins extractable from dried aster roots is very low (250 mg from 5-10 kg of dried roots), it is desirable to find ways to improve the formation of astins. For this purpose, it is a prerequisite to first understand the biosynthetic pathway of astin formation. Since astins contain some non-proteinogenic amino acids, it can be assumed that they are synthesized via non-ribosomal peptide synthesis. Most astins also contain a chlorinated proline-derived residue and cyclization, which, like chlorination, are very important for anti-tumor activity. The incorporation of the chlorine atoms is assumed to result from catalyzation by a flavin-dependent halogenase.

The aim of this project was therefore to identify the genes required for astin biosynthesis in Aster tataricus, the cloning of these genes and their expression in various hosts to improve astin production.

For the detection of the biosynthetic genes that could not be expected to be clustered in the plant, probes and primers had to be developed for the individual genes.

Since the consortium consisted of experts in the fields of non-ribosomal peptide synthesis, flavoenzymes, plant secondary metabolites and biological halogenation, the individual partners of the consortium used specific probes/primers and screened the plant genomic DNA for specific genes.

A metabolite with very high structural similarity to astins, cyclochlorotine, was isolated from the rice-spoiling fungus Penicillium islandicum. In contrast to astins, cyclochlorotine is described as a hepatotoxic compound. Based on the high structural similar-



Chemical structure of astin C

ity of the two compounds, we assumed that the biosyntheses would also be almost identical and thus involve highly similar genes and enzymes. Analysis of a large number of P. islandicum strains showed that all of them produce cyclochlorotine, but none of them produce astins. On the other hand, no P. islandicum strain could be isolated from dried or fresh A. tataricus roots, although we could, for the first time, detect astins in fresh A. tataricus roots. Additionally, none of the other fungi isolated from the surface of A. tataricus roots could be shown to produce astins or cyclochlorotine either.

Since we assumed that it would be easier to detect and clone the cyclochlorotine genes from the fungus P. islandicum than from the plant A. tataricus, we decided to sequence the P. islandicum genome with the help of the group of Jörn Kalinowski, Universität Bielefeld, Germany. The obtained sequence showed the presence of all the genes potentially required for the biosynthesis of cyclochlorotine. In some cases, several candidate genes could be detected. Using detailed bioinformatic analyses, it was possible to identify the most likely candidates for an involvement in cyclochlorotine biosynthesis.

For heterologous expression of the astin biosynthetic genes, we used several different hosts, including bacteria, yeast and hairy root cultures of A. tataricus. Attempts to obtain sterile A. tataricus plants using different methods failed. It turned out that a fungal endophyte was present in these plants, which could only be detected after several weeks of cultivation of the plants treated with HOCl or antibiotics for sterilization.

The fungal endophyte is a new fungus not previously described. Methods for cultivation of the fungus independently of A. tataricus were developed and it could be shown that this fungus is the actual producer of astins. However, it is possible that some of the modifications seen in some of the astins are introduced by the plant itself. For identification of the biosynthetic genes, the fungal genome was sequenced. Based on the sequences obtained, the genes can now be cloned and expressed.

The identification of the actual astin producer as a fungal endophyte of A. tataricus allows the production of astins by fermentation. As a result, larger amounts of astins are now available. Astin production could be even further improved by heterologous expression of the genes in suitable hosts. This would also open up the possibility to specifically obtain the most active astin and would allow the production of modified and improved astin derivatives with respect to their anti-tumor activity.

Impact

The identification of the producer of the anti-tumor compound astin as a fungus allows the production of larger amounts of these anti-tumor compounds by fermentation, instead of by extraction from dried aster roots, and makes possible their use for further development as an anti-tumor compound.

In addition, it raises the question of whether other health-promoting compounds derived from aster root extracts, as used in traditional Chinese medicine, are also produced by this fungus. If this should be the case, they could also be produced in larger amounts and could be specifically analyzed for the pharmacological properties. Using the fungus instead of the plant as the cell factory will avoid the use of herbicides, fertilizers and additional cultivation area.