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

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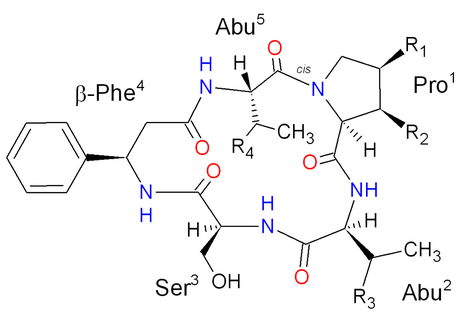
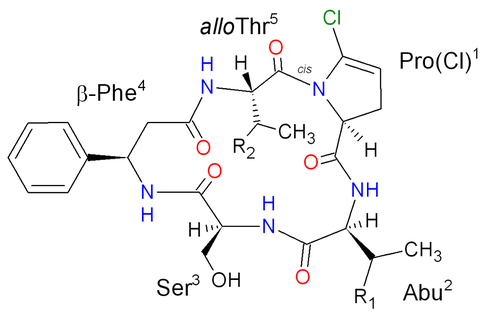
Aster tataricus is a plant native to Siberia, Japan and Northern China. The roots of *Aster tataricus* are traditionally used in Chinese medicinal herb teas due to antibacterial and antiviral activities of compounds present in these roots. Amongst the secondary metabolites which have been isolated from these roots are astins and astin derivatives. Astins have been shown to have anti-tumour activity indicating a use in cancer therapy. However, only very low amounts of astins can be isolated from the plant. It is also difficult to synthesise them due to several unusual features in the molecule and chemical synthesis produces also unwanted byproducts with environmental impacts.
Astins are dichlorinated, cyclic pentapeptides containing non-proteinogenic amino acids indicating a biosynthesis via non-ribosomal peptide synthetases (NRPS) and the involvement of halogenases.
The aim of the project is to detect the genes of the astin biosynthesis "gene cluster" and to use these genes to enhance astin production in *in vitro* cultures like hairy roots or callus.

1. Astins and astin derivatives



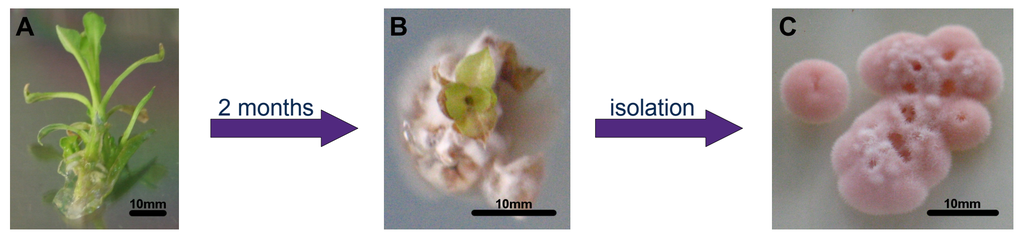


astin	R_1	R_2	R_3	R_4
A	Cl	Cl	H	OH
B	Cl	Cl	OH	H
C	Cl	Cl	H	H
F	H	Cl	H	H
G	H	H	H	H
I	Cl	OH	H	H

astin	R_1	R_2
D	H	H
E	OH	H
H	H	OH

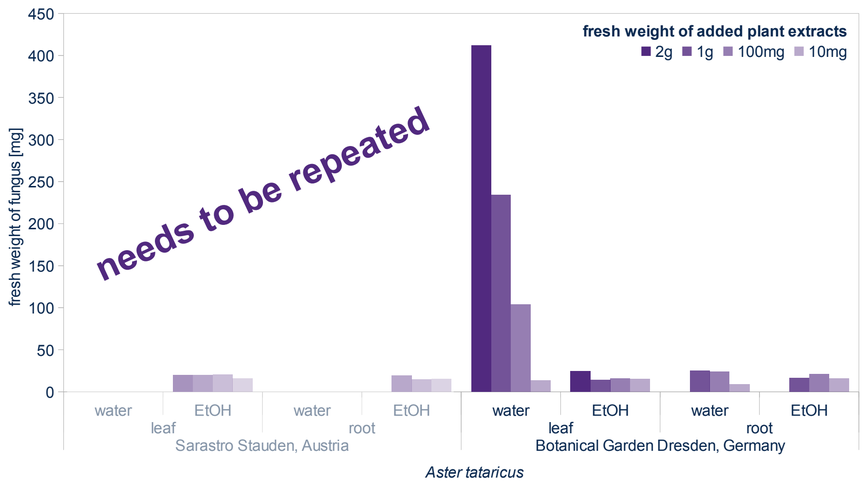
The astins and astin derivatives are characterised by a 16-membered ring system containing proteinogenic (proline and serine) and non-proteinogenic (β-amino phenylalanine, α-amino butyric acid and *allo*threonine) amino acids [1]. These non-proteinogenic amino acids strongly suggest a non-ribosomal peptide synthetase yet not described in plants at all. A second very unusual amino acid derivative is the chlorinated proline for which a halogenase is required. Only astins with the C,N-ring system and with the dichlorinated proline show the anti-tumour activity [2, 3].

2. *in vitro* cultivation of *Aster tataricus*



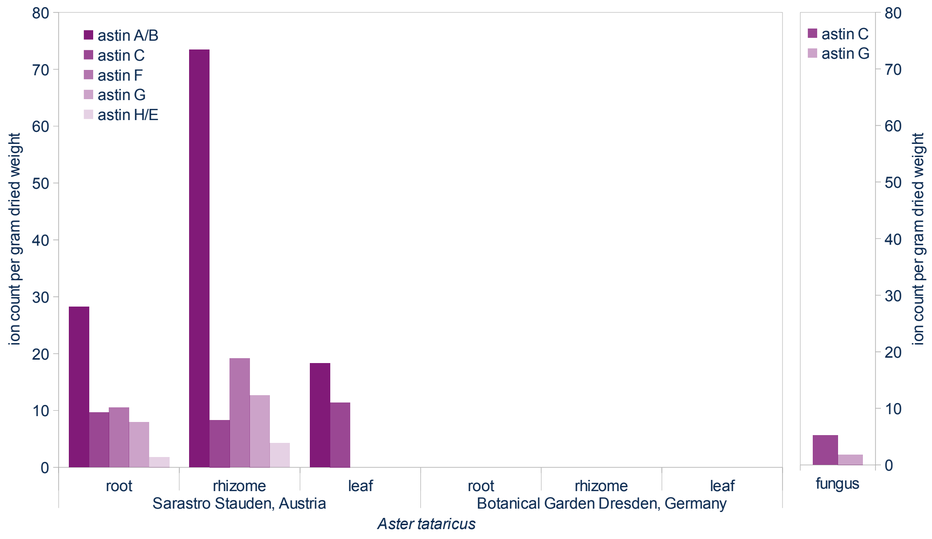
To get *in vitro* plants the inflorescence axis of *Aster tataricus* (Sarastro Stauden, Austria) was cut, sterilised and then grown on MS medium with auxin and cytokinin. The *in vitro* plants were received after 3 to 4 months cultivation (**A**). After additional 2 months a few fungi occurred on the plants which did not belong to a secondary contamination (**B**). Despite several treatments with different antimycotics one fungus still occurred in the cultures which was then isolated and cultivated (**C**). This undescribed fungus showed 85-90% identity with other fungi from the Pezizomycotina, Ascomycota.

3. Growth of the unknown fungus



Growth of the unknown fungus with different extracts of two *Aster tataricus* varieties.
The fungus was grown in liquid PDB (28°C, 200rpm) with extracts of the plant organs which were made with water or ethanol.
The fungal growth is enhanced by the addition of a water extract from the leaves of *Aster tataricus* deriving from the Botanical Garden Dresden, Germany.

4. Production of astins in the plant and in the fungus



Concentration of different astins in the *Aster tataricus* varieties and the unknown fungus. The plants were grown in soil in a climate chamber under long day conditions. The fungus was grown in PDB (28°C, 200rpm) for 50 days. Analysis was done by HPLC-MS.
No detectable astins were found in the *Aster tataricus* (Botanical Garden Dresden, Germany). The roots and rhizomes from the *Aster tataricus* (Sarastro Stauden, Austria) show a much larger spectrum of detectable astins than the fungus.
A MS-MS of the astins revealed that the astins C and G of the fungus are exactly the same as the astins C and G found in *Aster tataricus* (Sarastro Stauden, Austria).

Conclusion & future work

The fungus produces astins C and G and lives as endophyte in the plant.

➔ Localisation of the fungus in the plant via PCR.
➔ Cocultivation of *Aster tataricus* (Botanical Garden Dresden, Germany) and the fungus to look for astin production in the plant and/or fungus.
➔ Generating *in vitro* plants of *Aster tataricus* (Sarastro Stauden, Austria) without the fungus.

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References & acknowledgements

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