TECHNISCHE UNIVERSITÄT DRESDEN

Faculty of Science, Department of Biology, Institute of Botany, Chair of Plant Physiology

An endophytic fungus as silent astin producer in *Aster tataricus*?

Linda Jahn[1], Thomas Schafhauser[2] and Jutta Ludwig-Müller[1]

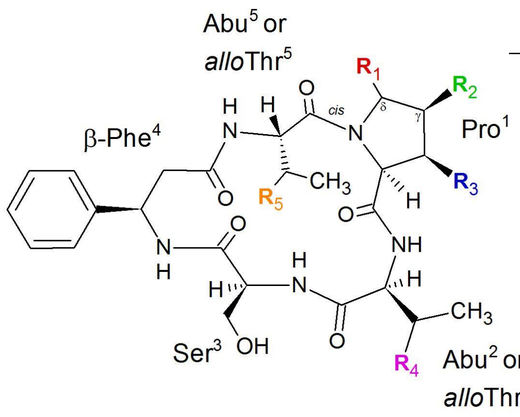
[1]TU Dresden, Faculty of Science, Institute of Botany, 01217 Dresden, Germany. Contact: Linda.Jahn@tu-dresden.de

[2] University of Tübingen, Department of Microbiology/Biotechnology, 72076 Tübingen, Germany

Aster tataricus is a plant (family: Asteraceae) originated in the North-Eastern part of Asia. Its roots are well known in the Traditional Chinese and Japanese Medicine as herbal tea. The root extract possesses antibacterial, antiviral, antiulcer and diuretic activities caused by the different secondary metabolites. One class of these metabolites are astins – chlorinated, cyclic pentapeptides consisting of proteinogenic and non-proteinogenic amino acids – found in the early 1990s. Since then, over 15 different astins were discovered yet (Morita et al. 1996, Xu et al. 2013). A few of them show an anti-tumor activity which depends on the cyclic structure and the *cis*-dichlorinated proline (Rossi et al. 2004). The concentration of astins is very low in roots, only a few milligrams could be isolated from 10kg of dried roots by Morita and colleagues.
We isolated an endophytic fungus from the inflorescence axis of *Aster tataricus* which is capable to synthesize astins and might be an alternative as astin producer. We show the difference in the astin production in the fungus and in the plant along with the distribution of the fungus and the astins in the plant *Aster tataricus*.

1. Astins and their derivatives

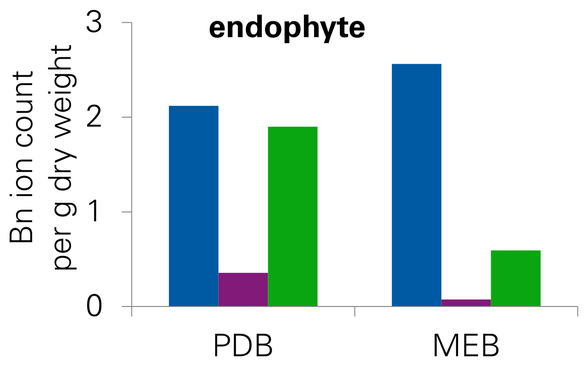
astin	R_1	R_2	R_3	R_4	R_5	γ to δ
A	H	Cl	Cl	H	OH	CH-CH
B	H	Cl	Cl	OH	H	CH-CH
C	H	Cl	Cl	H	H	CH-CH
F	H	H	Cl	H	H	CH-CH
G	H	H	H	H	H	CH-CH
I	H	Cl	OH	H	H	CH-CH
K	H	Cl	Cl	OH	OH	CH-CH
M	H	Cl	H	H	H	CH-CH



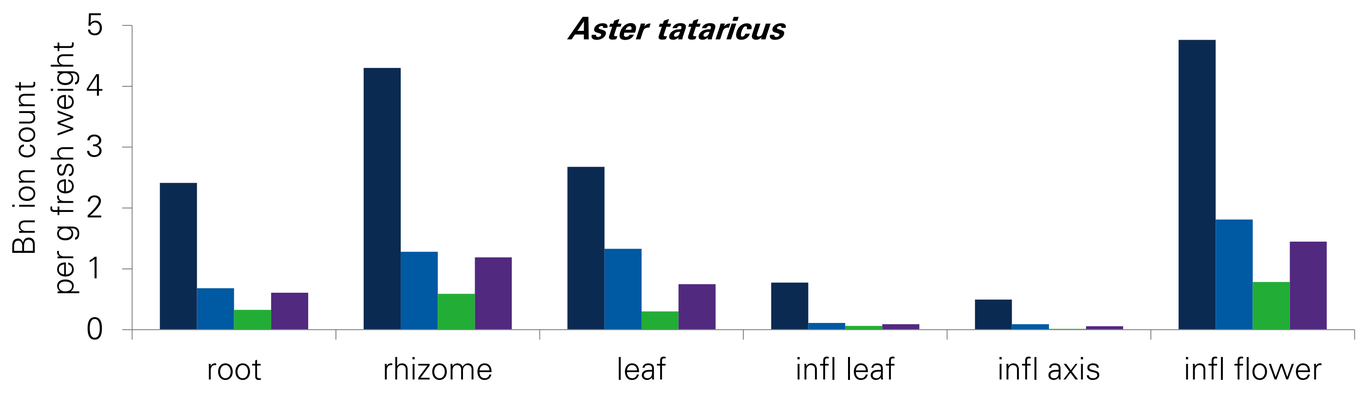
astin	R_1	R_2	R_3	R_4	R_5	γ to δ
D	Cl	H	H	H	H	C=C
E	Cl	H	H	OH	H	C=C
H	Cl	H	H	H	OH	C=C
L	Cl	H	H	OH	OH	C=C
N	H	Cl	H	H	H	C=C

- ✓ cyclic structure of
  - ✓ proteinogenic (proline and serine) and
  - ✓ non-proteinogenic amino acids (β-amino phenylalanine, α-amino butyric acid and *allo*threonine)
- ✓ dichlorination of *cis*-proline → important for anti-tumor activity

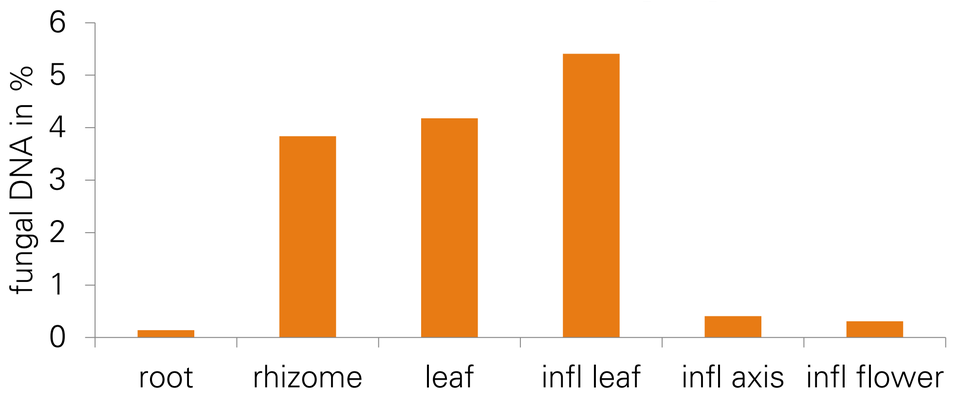
2. Astin spectrum in *Aster tataricus* and the endophyte



Astin measurements (HPLC-MS) **in *Aster tataricus* and the isolated endophyte**. Both show different astin spectra: The endophyte produces astins C, F and G, whereas *Aster tataricus* contains more astin variants.
(PDB potato dextrose broth, MEB malt extract broth, astin ■A/B ■C ■F ■G ■M)

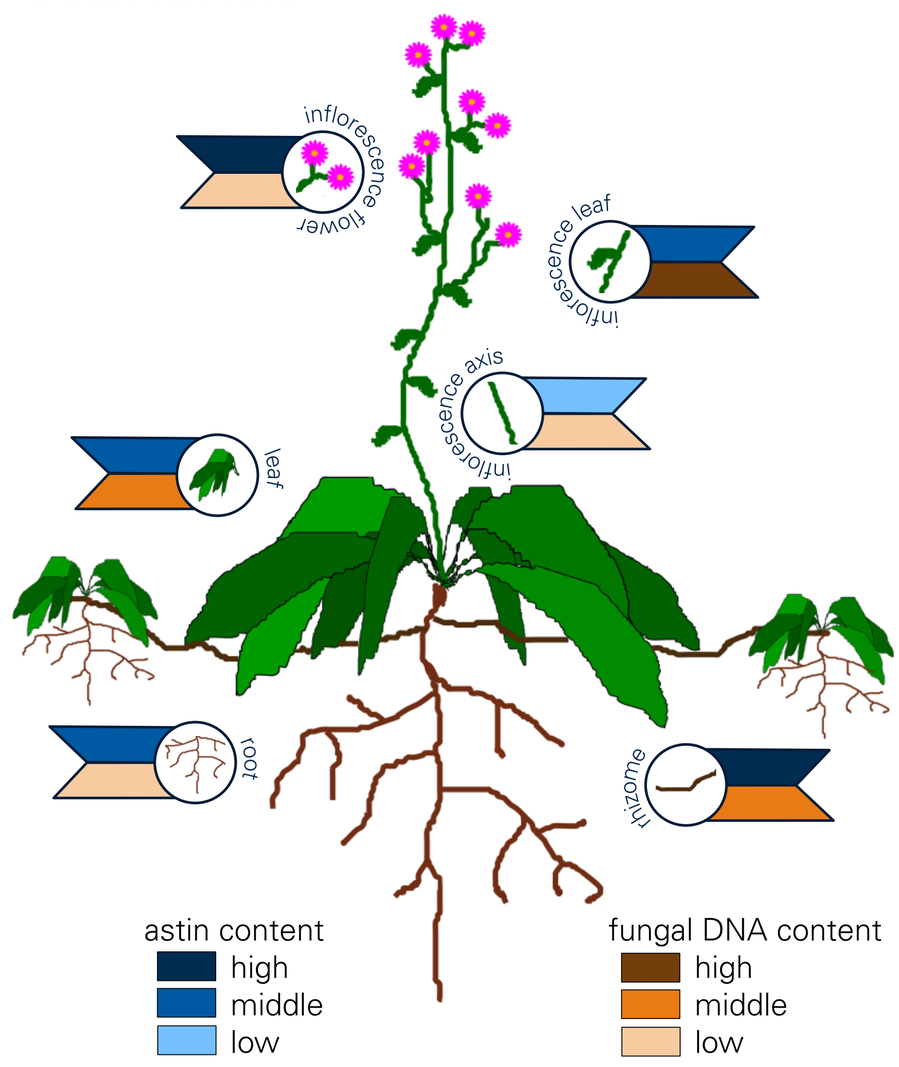


3. Distribution of the endophyte in *Aster tataricus*



Ratio of fungal to plant DNA. The highest content of fungal DNA was present in the green organs leaf and inflorescence leaf as well as in the rhizome.

Conclusion



- different astins in endophyte and plant
  → modification of fungal astins in plant?
- no linear correlation between astin content and fungal DNA
  → transport of astins from source to sink?

Future work

Increasing the astin production of the endophyte in culture

- by improving the growth conditions and
- by homologous as well as heterologous expression of biosynthetic genes.

Detection of the endophyte in the plant with the microscope

- by GFP labeling and
- by GUS labeling.

Europa fördert Sachsen. EFRE Europäischer Fonds für regionale Entwicklung. Europäische Union

ERA IB European Research Area Industrial Biotechnology

References

[1] Morita *et al*. (1996). *Chemical & Pharmceutical Bulletin*, 44: 1026-1032.

[2] Rossi *et al*. (2004). *Journal of Peptide Science*, 10: 92-102.

[3] Xu *et al*. (2013). *Tetrahedron*, 69: 7964-7969.