The identification of tetrahydrocannabinolic acid synthase in the non-aqueous secretions of the storage cavities from *Cannabis sativa* glandular trichomes

Pawel Rodziewicz¹, Stefan Loroch², Ingo Feldmann², Cornelia Schumbritzki², and Oliver Kayser¹

¹Technical Universität Dortmund, Fakultät Bio- und Chemieingenieurwesen, Technische Biochemie
²Leibniz-Institut für Analytische Wissenschaften – ISAS – e.V.
pawel.rodziewicz@tu-dortmund.de, stefan.loroch@isas.de, ingo.feldmann@isas.de, cornelia.schumbritzki@isas.de, oliver.kayser@tu-dortmund.de

**INTRODUCTION**

*Cannabis sativa* L. is an important herbaceous species cultivated since ancient times due to its unique medicinal and recreational properties, but also as a source of valuable seed oil and high quality fibre. Cannabinoids represent the unique class of terpenophenolics, which largely contribute to the pharmacological properties of this species. More than 100 cannabinoids is known, but Δ⁹-tetrahydrocannabinolic acid (THCA), cannabiolic acid (CBDA) and cannabichromenic acid (CBCA) are produced in the largest quantities in this plant (Fig. 1). Cannabinoids are synthesized in glandular trichomes present mainly on female flowers and their main reservoirs are storage cavities of these hair-like structures (Fig. 2) [1].

Tetrahydrocannabinolic acid synthase (THCA synthase) catalyses the unique oxidative cyclization of cannabigerolic acid (CBGA) into THCA (Fig. 1), which is a direct precursor of the mind-affecting cannabinoids. THCA synthase gene consists of a 1635-nucleotide open reading frame, encoding a 546-amino acid polypeptide and theoretical mass 62 kDa. However, the reported mass value obtained by SDS-PAGE of the purified enzyme was higher indicating that THCA synthase undergoes post-translational modifications, e.g. glycosylation. The first 28 amino acid residues constitute the signal peptide for which secretory pathway was predicted [2]. It has already been suggested that THCA synthase is secreted into storage cavities of glandular trichomes, where it might also synthesize the final product – THCA [3]. However, no direct evidence on protein level has yet been presented.

**METHODS**

Leaves containing glandular trichomes (Fig. 2) of drug type *Cannabis sativa* L. at the 7th week of flowering stage were used for experiments. Secretions from trichome storage cavities were extracted using thin capillary attached to the cell manipulator TransferMan 2 (Eppendorf, Germany) (Fig. 3). After microsuction of every 10 storage cavities the collected material was extracted using thin capillary attached to the cell micromanipulator TransferMan 2 (Eppendorf, Germany) (Fig. 3). After microsuction of every 10 storage cavities the collected material was transparently dyed using silver ion-trap-Orbitrap Mass Spectrometer coupled to UltiMate™ 3000 RSLCnano System (Thermo Fisher Scientific, USA). Prior analysis a blank-MS-run was acquired to identify mass spectrum only from the sample containing 800 trichomes. A few cellular proteins were concomitantly identified, but appeared to be of very low abundance (Tab. 1). To confirm the identification of THCA synthase in storage cavity secretions western blot analysis was also applied. Using antibody specific for THCA synthase we were able to detect a signal below 70 kDa, corresponding to the protein band on the SDS gel identified as THCA synthase (Fig. 5).

**RESULTS**

During detection of low density of Coassme staining, protein bands were visualized only for samples containing 400 and 800 secretions (Fig. 4). However, the THCA synthase was clearly identified by mass spectrometry only from the sample containing 800 trichomes. A few cellular proteins were concomitantly identified, but appeared to be of very low abundance (Tab. 1). To confirm the identification of THCA synthase in storage cavity secretions western blot analysis was also applied. Using antibody specific for THCA synthase we were able to detect a signal below 70 kDa, corresponding to the protein band on the SDS gel identified as THCA synthase (Fig. 5).

**CONCLUSIONS AND FUTURE PROSPSPECTS**

In this study for the first time the extracellular presence of THCA synthase in the non-aqueous secretions of storage cavities from glandular trichomes was confirmed on the protein level both by mass spectrometry and western blot analysis. However, the identification of the enzyme was only possible when a large number of secretions was analysed.

The subsequent experiments will concern monitoring of the THCA synthase abundance over flowering period and development of the assay tests to examine the THCA synthase activity in the non-aqueous environment. In the next steps we will also conduct similar analysis on the CBDA-rich non-drug type cannabis plants ( hemp) to investigate the potential extracellular localization of the CBDA synthase.

**REFERENCES**

6. Pawel, rodziewicz@tu-dortmund.de, stefan.loroch@isas.de, ingo.feldmann@isas.de, cornelia.schumbritzki@isas.de, oliver.kayser@tu-dortmund.de