

# Molecular-ecological Studies on Bile Acid Degrading Bacteria in Soil for Assessment of Plant Protection Products

Antonia Bruder<sup>1,2</sup>, Julio Cesar Coutiño-Montes<sup>2</sup>, Karsten Schlich<sup>1</sup>, Cecilia Andrea Diaz Navarrete<sup>1</sup>, Bodo Philipp<sup>1,2</sup>

<sup>1</sup> Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Schmallenberg, Germany

<sup>2</sup> Institute for Molecular Microbiology and Biotechnology, University Münster, Münster, Germany

contact: bodo.philipp@ime.fraunhofer.de; antonia.bruder@ime.fraunhofer.de

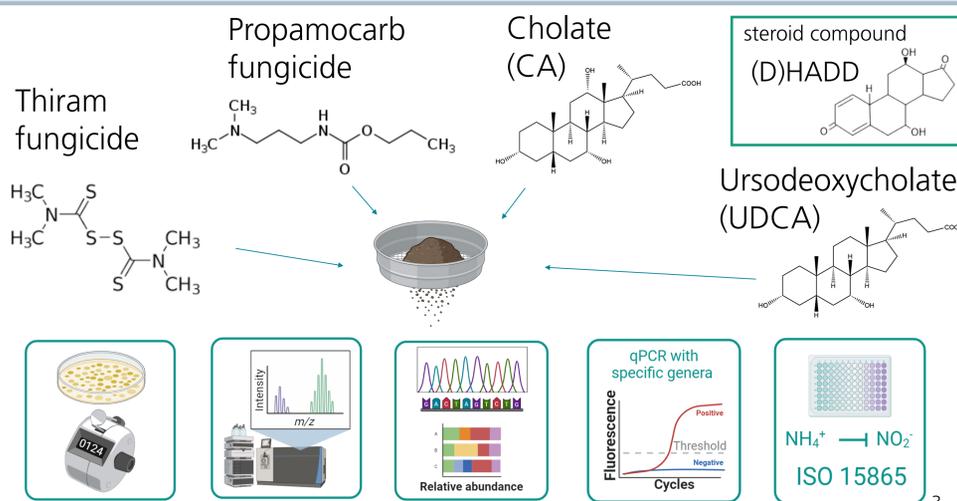
## Introduction

- The metabolic performance of soil bacteria is primarily assessed by nitrification<sup>1</sup>, but changes in microbial communities following the introduction of substances are not considered.
- Specific carbon and energy sources, which are only used as substrates by certain taxa, could be used to correlate metabolic activity with the structure of the soil microbial community.
- Bile acids, released by all vertebrates, are degraded by a limited number of bacterial genera such as *Comamonas*, *Pseudomonas*, *Rhodococcus*, or bacteria from the family *Sphingomonadaceae*<sup>2</sup>.
- Possibility to assess the functional capacity of the microbial metabolism of bile acids by specific bacterial taxa in the soil to provide information on the effects of plant protection products (PPP).

## Aim of the study

Development of methods to measure soil bile acid degradation and identify related bacteria, then studying how pesticide pollution affects this microbial community's ability to degrade bile acids.

## Material & Methods



## Results

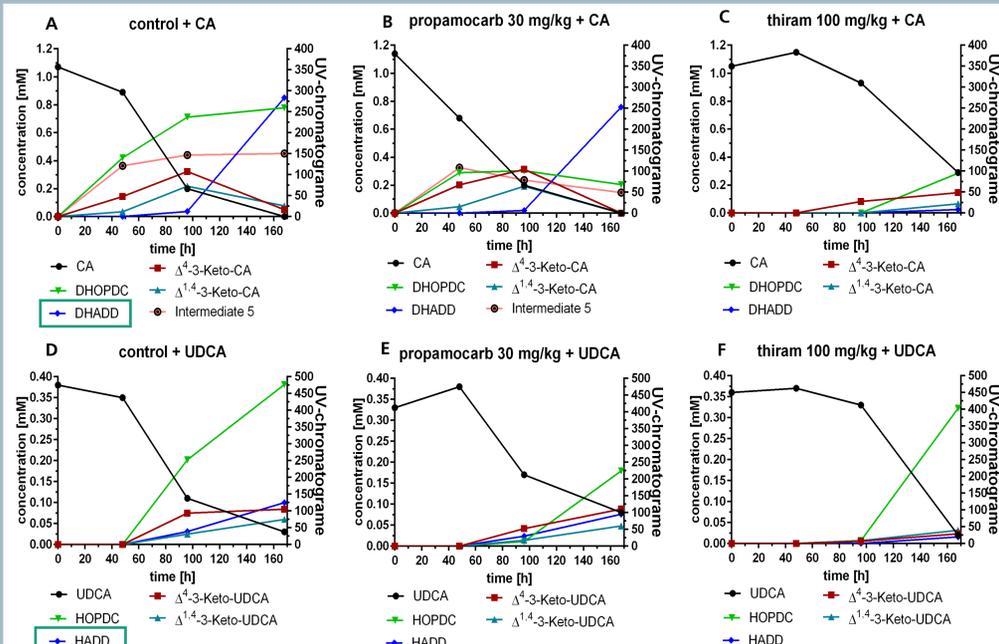


Figure 1: HPLC-MS analyses of soil samples containing bile acids with PPP. DHOPDC/HOPDC (7,12-(di)hydroxy-3-oxo-pregna-1,4-diene-carboxylate), DHADD/HADD (7 $\alpha$ ,12 $\alpha$ / $\beta$ -(di)hydroxy-androsta-1,4-diene-3,17-dione).

## Results

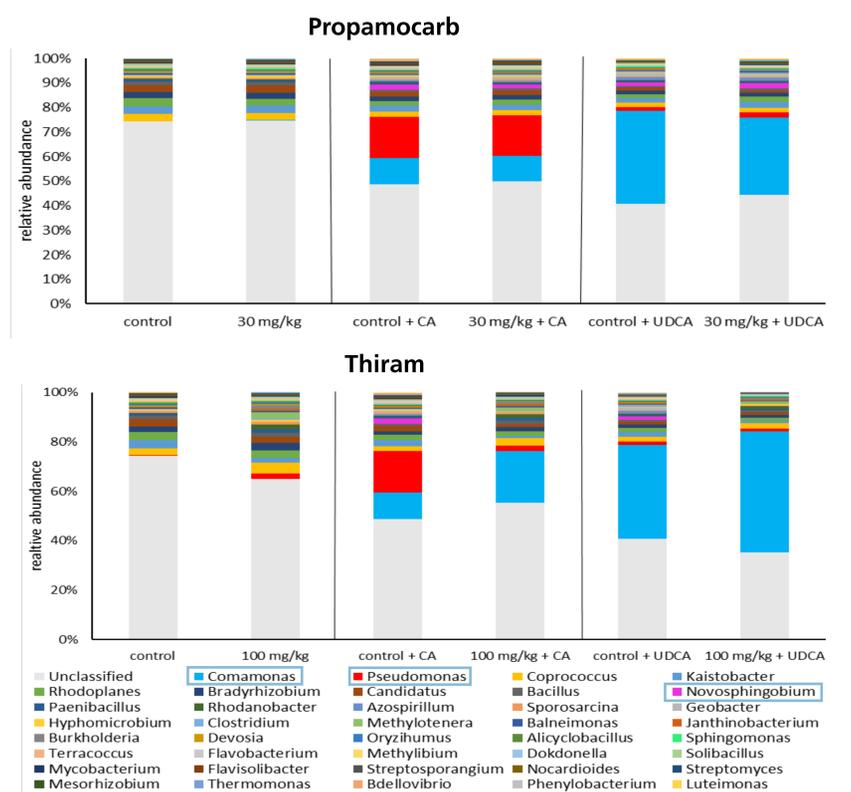


Figure 2: 16S rRNA metabarcoding of soil samples with PPP and bile acids. Relative abundance of the 45 most dominant genera.

## qPCR analysis of the specific genera *Pseudomonas* and *Comamonas*

- Inhibition of *Pseudomonas* (Thiram + CA) and *Comamonas* (Propamocarb + UDCA), was observed with both, qPCR and Metabarcoding, consistently.

## Cultivation of bile acid-degrading soil bacteria

- Significantly increased growth of bile acid-degrading bacteria in soils spiked with the CA and UDCA.
- Propamocarb:** Slight inhibition of UDCA-degrading bacteria growth.
- Thiram:** Significantly inhibited growth of CA degrading bacteria with, clear tendency with UDCA-degrading bacteria.

## Conclusion

- ISO15865:** Inhibition of nitrification compared to control: Propamocarb: 19%, Thiram: 100%
- Minimal inhibition of propamocarb on nitrification and bile acid degradation
- High inhibition of thiram on nitrification and bile acid degradation
- Functionality of bile acid degradation in soil could serve as a parameter for analysing the effects of PPP on soil microorganisms**
- considers not only impairment of function but also the associated taxa
- **Structure-function correlation**

## Outlook

- Repetitions with different soils and other PPP, veterinary drugs or other relevant substances
- Consider qPCR analysis with less robust genera
- To quantify only bacteria relevant in bile acid degradation, use already known and important genes for bile acid degradation
- Accumulation of **steroid compounds** that may be present extracellularly during bacterial degradation → Impairment of other important soil organisms<sup>4</sup>



<sup>1</sup> OECD-Guideline for the Testing of Chemicals, Soil Microorganisms: Nitrogen Transformation Test, Guideline 216, January 21, 2000.

<sup>2</sup> Feller, Franziska Maria; Holert, Johannes; Yücel, Onur; Philipp, Bodo (2021b): Degradation of Bile Acids by Soil and Water Bacteria. In: *Microorganisms* 9 (8). DOI: 10.3390/microorganisms9081759.

<sup>3</sup> All figures in Material & Methods are created with Biorender.com

<sup>4</sup> Mendelski, M. N.; Dölling, R.; Feller, F. M.; Hoffmann, D.; Ramos Fangmeier, L.; Ludwig, K. C. et al., (2019): Steroids originating from bacterial bile acid degradation affect *Caenorhabditis elegans* and indicate potential risks for the fauna of manured soils. In: *Scientific reports* 9 (1), S. 11120. DOI: 10.1038/s41598-019-47476-y.