RUB

Applications are welcome for **11 PhD positions** in the Research Training Group **Microbial Substrate Conversion**

The RTG **Microbial Substrate Conversion** (MiCon) aims at understanding the mechanistic principles of microbial metabolic processes at the molecular level. Many of the investigated enzymes and pathways are not only of fundamental interest but also attractive for biotechnological and medical applications. Major topics are:

- Conversion of small inorganic compounds
- Biosynthesis of specialized metabolites
- Selective (chemo-)enzymatic conversion of small organic substrates

We offer an interdisciplinary training program tailored to the individual need of the doctoral researchers. Disciplines involved are Microbiology, Biochemistry, Chemistry, Biophysics, Biotechnology and Plant Sciences.

Further information on the individual projects and the qualification program can be found on our website: www.rub.de/micon.

Please submit your application until January 21, 2024 as single PDF including CV, degree certificates, one-page abstract of your Master thesis, motivation letter and your preference for up to three of the available projects to mikrobiologie@rub.de.

The positions are funded for three years, starting July 1st, 2024. An earlier or later start can be arranged in individual cases. Travel expenses for interviews can be refunded.

Sincerely

(Prof. Dr. Franz Narberhaus - Speaker)

RUHR UNIVERSITÄT BOCHUM
Research Training Group 2341
Microbial Substrate Conversion

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Project Ute Krämer

Biosynthesis of the natural high-affinity metal chelator nicotianamine with applications in biofortification and medicine

Background and preliminary work: Nicotianamine (NA), a high-affinity metal chelator and low-molecular-mass non-proteinogenic amino acid, is a well-known natural product of flowering plants. NICOTIANAMINE SYNTHASE (NAS) proteins, first discovered in tomato and barley, catalyse the biosynthesis of NA from three molecules of S-adenosylmethionine (SAM).

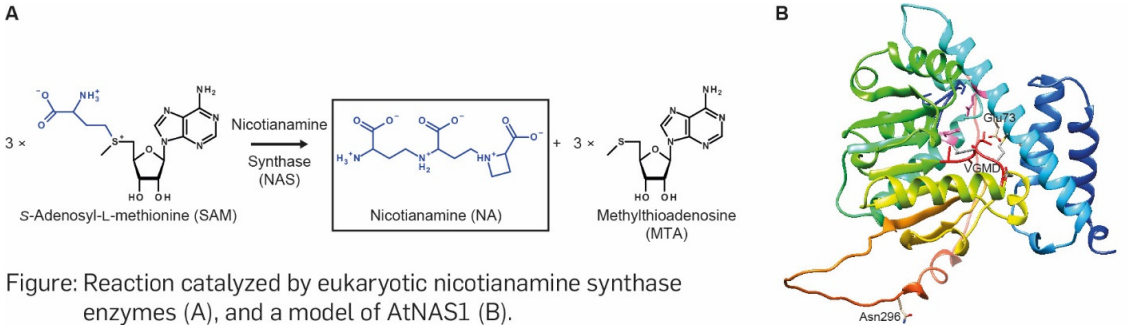

Figure: Reaction catalyzed by eukaryotic nicotianamine synthase enzymes (A), and a model of AtNAS1 (B).

Long after the identification of NAS, similar proteins were discovered in other organisms: NA is also synthesized by some filamentous fungi, and NA-related chelators are produced by some archaea and bacteria. Indispensable in flowering plants, NA maintains the inter-cellular mobility of the nutrient metals, for example iron and zinc (Zn), and NA-derived molecules named phytosiderophores are required for root iron uptake by YELLOW STRIPE-LIKE transporters in cereals. NA has become a prime target compound in crop biofortification towards combating human malnutrition, and it also possesses anti-hypertensive pharmacological activity.
NAS and NAS-related proteins catalyse several consecutive reactions in a single active site, as was concluded based on crystal structures of an NAS-like archaeal thermoNAS. Its reaction product thermoNA contains a glutamate instead of the terminal SAM-derived azetidine-2-carboxylate moiety characteristic of NA. After establishing a quantitative NAS enzyme assay, the Krämer group discovered that NAS proteins of flowering plants consist of a core-NAS domain and a C-terminal extension that is autoinhibitory *in vitro*. They also pinpointed a number of essential residues at the active site of *Arabidopsis thaliana* NAS1. Earlier, the group showed that the Arabidopsis membrane transport protein ZINC-INDUCED FACILITATOR 1 (ZIF1) mediates the vacuolar sequestration of NA, which functions to control sub-cellular and whole-plant Zn partitioning. Interestingly, no proteins acting in the catabolism of NA are known. A possible NA breakdown product – the toxic proline analogue and allelopathic chemical azetidine-2-carboxylate – was detected in only a few plants to date.

Work planned: The proposed project will address **structure-function relationships of fungal and plant NAS proteins** and **explore avenues for enhancing cellular NA production**.
(1) Our first goal is to identify residues of *Neurospora crassa* NAS required for intermediate and final product formation during catalysis, including the formation of the azetidine ring that is exceedingly rare in biological chemistry. To this end, we will combine site-directed mutagenesis, biochemical *in vitro* and *in vivo* activity assays, and LC-mass spectrometry-based identification of products (cooperation with Frank Schulz), based on protein structural modelling using alphafold2 and phyre2 (cooperation with Eckhard Hofmann; see Figure above).
(2) Our second goal is to understand the mechanisms underlying NAS autoinhibition and its release. Following up on our previous observations, we will use site-directed mutagenesis to comprehensively map all amino acids required for autoinhibition of AtNAS1 and AtNAS4 in the C-terminal extensions as well as on the surface of the core-NAS domains. Furthermore, we will employ sequence comparisons and amino acid/domain swaps with NcNAS. Biochemical activities and properties of the C-terminal peptides will be analysed using microscale thermophoresis, and Inductively-Coupled Plasma Optical Emission and Mass Spectrometry (ICP-OES, ICP-MS) for the quantification of bound metal cations, for instance. Both project parts

will involve the use of heterologous yeast (*Saccharomyces cerevisiae*) and homologous plant expression systems.

Selected references:

Dreyfus C, Lemaire D, Mari S, Pignol D, & Arnoux P (2009) Crystallographic snapshots of iterative substrate translocations during nicotianamine synthesis in Archaea. *Proc Natl Acad Sci USA* 106:16180-16184.

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Haydon MJ*, et al.* (2012) Vacuolar nicotianamine has critical and distinct roles under iron deficiency and for zinc sequestration in Arabidopsis. *Plant Cell* 24:724-737.

Seebach H, Radow G, Brunek M, Schulz F, Piotrowski M, Krämer U (2023) Arabidopsis nicotianamine syntheases comprise a common core-NAS domain fused to a variable autoinhibitory C terminus. *J Biol Chem* 299: 104732.