

## Lab project / Bachelor or Master's thesis at the Institute of Molecular Biotechnology

Cloning and characterization of undiscovered N-hydroxylating and N-N-bond forming enzymes for their application in biocatalysis

### DESCRIPTION:

N–N bonds have only been identified in approximately 200 natural products. These compounds display remarkable structural diversity and have a wide variety of biological activities. Despite the identification of biosynthetic gene clusters for a number of compounds with N–N bonds, only one enzyme that catalyzes N–N bond formation in these naturally occurring organic metabolites has been reported so far. In the same gene clusters, N-hydroxylating enzymes have been identified, but have hardly been characterized and applied in biocatalysis so far. The objective of this project is the cloning of several N-hydroxylating and N-N-bond forming enzymes into *E. coli*. For this, codon-optimized synthetic genes will be cloned into several expression vectors and transformed into *E. coli*. The expression levels will be investigated and, if necessary, optimized. In the second part of the project, GC and HPLC analysis will be established and the enzymes will be applied in several biocatalytic reactions. The long-term objective of this master's thesis is the better understanding of these highly interesting enzymes and should pave the way to an application in biocatalysis.

### TASKS:

- Cloning of synthetic and codon-optimized genes as fusion to a C-or N-terminal hexahistidine-tag in expression vectors
- Verification of the functional expression of the enzymes with SDS-PAGE and Western-blot
- Biocatalytic reactions either with isolated enzymes or whole-cell catalysis

### REQUIREMENTS:

Practical experience in molecular biology is desirable, but high motivation and a pro-active personality is more important.

### STARTING DATE:

Negotiable.

### CONTACT PERSON:

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