

University of Groningen

Biochemical and functional characterization of Nudix hydrolase enzymes with novel regulatory roles in Gram positive methylotrophic bacteria

Kloosterman, Harmen

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version

Publisher's PDF, also known as Version of record

Publication date:

2005

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Kloosterman, H. (2005). *Biochemical and functional characterization of Nudix hydrolase enzymes with novel regulatory roles in Gram positive methylotrophic bacteria*. s.n.

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

**Biochemical and functional characterization of Nudix hydrolase
enzymes with novel regulatory roles in Gram positive
methylophilic bacteria**

Harm Kloosterman

The study described in this thesis was performed at the Department of Microbiology, Groningen Biomolecular Sciences and Biotechnology Institute (GBB), University of Groningen, The Netherlands.

The research was supported in part by TNO Environmental Research.

RIJKSUNIVERSITEIT GRONINGEN

**BIOCHEMICAL AND FUNCTIONAL CHARACTERIZATION OF NUDIX
HYDROLASE ENZYMES WITH NOVEL REGULATORY ROLES IN GRAM
POSITIVE METHYLOTROPHIC BACTERIA**

Proefschrift

ter verkrijging van het doctoraat in de
Wiskunde en Natuurwetenschappen
aan de Rijksuniversiteit Groningen
op gezag van de
Rector Magnificus, dr. F. Zwarts,
in het openbaar te verdedigen op
vrijdag 28 oktober 2005
om 14.45 uur

door

Harmen Kloosterman

geboren op 6 juli 1964
te Garijp

Promotor: Prof. Dr. L. Dijkhuizen

Beoordelingscommissie: Prof. Dr. A.J. Driessen

Prof. Dr. J.D. van Elsas

Prof. Dr. J. Kok

Contents

| | | |
|------------------|--|-----|
| | Aim and outline of thesis | 7 |
| Chapter 1 | General introduction into Nudix hydrolase proteins | 11 |
| Chapter 2 | Nicotinoprotein methanol dehydrogenase enzymes in Gram-positive methylotrophic bacteria | 25 |
| Chapter 3 | Molecular, biochemical and functional characterization of a Nudix hydrolase protein that stimulates activity of a nicotinoprotein alcohol dehydrogenase. | 33 |
| Chapter 4 | Identification of a magnesium-dependent NAD(P)(H) binding domain in the nicotinoprotein methanol dehydrogenase from <i>Bacillus methanolicus</i> | 53 |
| Chapter 5 | A Nudix hydrolase protein with a crucial role in regulation of <i>Amycolatopsis methanolica</i> plasmid pMEA300 encoded functions | 69 |
| Chapter 6 | Identification and characterisation of the minimal replicon of the indigenous plasmid pMEA300 of the actinomycete <i>Amycolatopsis methanolica</i> | 85 |
| Chapter 7 | (De)Regulation of key enzyme steps in the shikimate pathway and phenylalanine specific pathway of the actinomycete <i>Amycolatopsis methanolica</i> | 97 |
| Chapter 8 | Summary and concluding remarks | 115 |
| Chapter 9 | Samenvatting | 125 |
| | References | 133 |
| | Dankwoord | 149 |

Aim and Outline of Thesis

At the start of this study no information was available about possible regulatory functions of Nudix (Nucleotide diphosphate linked to some other moiety, X) hydrolase proteins. This protein family was regarded as a group of enzymes necessary to remove potentially toxic or mutagenic compounds from the cell. This is exemplified by the first member of this protein family, MutT of *E. coli*, which was shown to hydrolyze 8-oxo-dGTP, a potentially mutagenic form of dGTP when used for DNA synthesis. The entire Nudix hydrolase protein family subsequently was referred to as “housecleaning enzymes”. The cloning and characterization of the methanol dehydrogenase (MDH) activator protein (ACT) of *Bacillus methanolicus* provided the first example of a Nudix hydrolase protein regulating an enzymatic activity involved in primary metabolism. Also a Nudix hydrolase encoding ORF on the indigenous plasmid pMEA300 of *Amycolatopsis methanolica* was shown to be an important regulator of various plasmid functions (pock formation, autonomous replication and stimulation of transformation frequency). The primary aim of this PhD study was to elucidate the physiological roles, regulatory functions and mechanisms of both Nudix hydrolases, which share significant overall amino acid sequence similarity.

Chapter 1 reviews the current knowledge of the Nudix hydrolase protein family, with emphasis on Nudix hydrolase proteins in microorganisms. Chapter 2 discusses the nicotinoprotein methanol dehydrogenase (MDH) enzymes in Gram-positive bacteria. The MDH enzymes in both *B. methanolicus* and *A. methanolica* are nicotinoproteins, with a tightly bound NAD(P)(H) cofactor. The *B. methanolicus* ACT protein hydrolyzes the NAD(H) cofactor of MDH (chapter 3). A detailed biochemical and mutational analysis of the catalytic function of this NAD(H) cofactor and its binding site in MDH are presented in chapter 4. Chapter 5 reports the identification of the minimal replicon of *A. methanolica* plasmid pMEA300, revealing that none of the regulatory genes -including the Nudix hydrolase encoding *orf192*- are essential for replication. The pMEA300 Rep protein shows no similarity with any other Rep protein in databases. The data suggest that pMEA300 belongs to a new family of Rolling Circle Replication plasmids. A functional analysis of the Nudix hydrolase Orf192 protein and other regulatory proteins of pMEA300 is described in chapter 6, revealing their effect on integration, pock formation and transformation frequency. Chapter 7 describes the regulation of key enzymes in the shikimate pathway of *A. methanolica*. Mutant enzymes affected in feedback inhibition/activation control were isolated by screening for mutant strains resistant to toxic phenylalanine analogs and subsequently characterized. Those mutants could only be isolated in the *A. methanolica* wild type strain, containing the indigenous plasmid pMEA300. No such mutants could be obtained in a strain devoid of pMEA300 sequences. Finally, chapters 8-9 summarize the results obtained, present concluding remarks and suggestions for further research.

