University of Groningen

ACV synthetase: a unique member of the nonribosomal peptide synthetases family

Iacovelli, Riccardo

DOI:
[10.33612/diss.156838414](https://doi.org/10.33612/diss.156838414)

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:
2021

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

Citation for published version (APA):
Iacovelli, R. (2021). *ACV synthetase: a unique member of the nonribosomal peptide synthetases family*. [Thesis fully internal (DIV), University of Groningen]. University of Groningen.
<https://doi.org/10.33612/diss.156838414>

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.

ACV synthetase: a unique member of the nonribosomal peptide synthetases family

Riccardo Iacovelli

The research described in this thesis was carried out in the Department of Molecular Microbiology, part of the Groningen Biomolecular Sciences and Biotechnology Institute (GBB), University of Groningen, The Netherlands. It was financially supported and funded by the Faculty of Science and Engineering, University of Groningen.

Cover: Lorenzo Arcangeli, [instagram.com/aar.lo/](https://www.instagram.com/aar.lo/)

Layout: Riccardo Iacovelli

Printed by: Gildeprint – Enschede, www.gildeprint.nl

Copyright © 2021 by Riccardo Iacovelli.

All rights reserved. No part of this thesis may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopy, recording or any information storage or retrieval system, without prior permission of the author.

university of
 groningen

ACV synthetase: a unique member of the nonribosomal peptide synthetases family

PhD thesis

to obtain the degree of PhD at the
 University of Groningen
 on the authority of the
 Rector Magnificus Prof. C. Wijmenga
 and in accordance with
 the decision by the College of Deans.

This thesis will be defended in public on

Friday 26 February 2021 at 16:15 hours

by

Riccardo Iacovelli

born on 7 November 1991
 in Tivoli, Italy

Supervisors

Prof. A.J.M. Driessen

Prof. R.A.L. Bovenberg

Assessment Committee

Prof. I.J. van der Klei

Prof. D.B. Janssen

Prof. R.P. de Vries

A mia madre e mio padre

Table of contents

Scope of the thesis	8
Chapter 1	11
Nonribosomal peptide synthetases: structure, function and biotechnological potential	
Chapter 2	59
Biochemical characterization of the <i>Nocardia lactamdurans</i> ACV synthetase	
Chapter 3	89
Investigations on the binding pocket of the L- α -aminoadipic acid-specific adenylation domain of the <i>Nocardia lactamdurans</i> ACV synthetase	
Chapter 4	111
Identification of a conserved N-terminal domain in the first module of ACV synthetases	
Chapter 5	145
Summary	146
Samenvatting	154
Riassunto breve	164
Appendix	169
Acknowledgements	170
About the author	176
List of publications	177

Scope of the thesis

The aim of this thesis was to investigate some of the fundamental aspects of substrate recognition and activation during the synthesis of nonribosomal peptides (NRP). In this thesis, the focus is on the enzyme ACV synthetase (ACVS), a unique trimodular nonribosomal peptide synthetase (NRPS) involved in the first step of the biosynthesis of β -lactam antibiotics.

Chapter 1 provides an overview of nonribosomal synthesis, including detailed descriptions of each of the structural domains that play key roles during the process, as well as the function of trans-acting proteins. Furthermore, the dynamics of the domain interactions during the catalytic process are outlined. Lastly, the biotechnological potential of NRPS is briefly discussed, with a particular focus on the history of engineering approaches and the latest progress in the field.

In **Chapter 2** we investigated the activity of the ACVS from the soil bacterium *Nocardia lactamdurans*. The protein can be very well overexpressed in *E. coli* and purified to near homogeneity, which allowed its characterization with respect to substrate specificity and product formation. Module 1 is strictly specific towards its substrate L- α -aminoadipic acid, while module 2 and 3 can activate a broader variety of amino acids.

Chapter 3 focuses on the interaction between the first adenylation domain (module 1) of the ACVS and its cognate substrate L- α -aminoadipic acid. The extraordinary activity of ACVS is that the first substrate L- α -aminoadipic acid is activated on its δ -carboxyl group, resulting in a noncanonical peptide bond with L-cysteine. Here, we generated a structural model and performed multiple sequence alignment analysis to investigate the architecture and chemistry of the binding pocket. This allowed the identification of certain residues with

potential key roles in substrate recognition and activation. To investigate this, we performed site-directed mutagenesis on those residues and analysed the impact of the mutations on ACV synthesis. The results suggest a non-canonical interaction between the binding pocket and the substrate.

Chapter 4 describes the discovery of a conserved domain in the first module of ACVS enzymes, previously unidentified. Bioinformatic analyses such as domain prediction and modelling revealed the presence of structured N-terminal regions in the first module of ACVS that show strong structural similarity with condensation (C) domains. However, these domains, referred to as C*, are about half the size of a full C domain and lack the canonical active site motif. To investigate their potential role, we generated deletion variants of *Penicillium rubens* and *Nocardia lactamdurans* ACVS and assessed the impact on the enzyme activity *in vivo* and *in vitro*, respectively. Both enzymes lost their ability to synthesize ACV, though we still observed the CV intermediate in the *in vitro* reaction, indicating that module 2 and 3 remain functional.

Chapter 5 briefly summarizes all the topics and findings discussed in the other chapters and provides a general outlook on the biotechnological potential of NRPS.