Itaconic acid production in *Aspergillus*

An Li
李安

"*Energy and persistence conquer all things.*"
—*Benjamin Franklin*

For my families and friends

ISBN: 978-7-5434-0570-7
Cover: Grand Universe
Printed by: HeBei Education Press Co., Ltd.

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Proefschrift

Ter verkrijging van
De graad van Doctor aan de Universiteit Leiden,
Op gezag van Rector Magnificus prof.mr. CJJ.M. Stolker,
volgens besluit van het College voor Promoties
te verdedigen op woensdag 20 november 2013
klokke 15:00 uur
door

An Li
李安

geboren te ShiJiazhuang, P.R. China
in 1982

Promotiecommissie

Promotor: Prof. Dr. P.J. Punt

Overige leden: Prof. Dr L. Diels, Universiteit van Antwerpen

Prof. Dr. A.J.M. Driessen, Universiteit Groningen

Dr Ir J. Urbanus, TNO

Prof. Dr C. J. ten Cate

Dr A. F. J. Ram

Prof. Dr. G. van Wezel

The work described in this thesis was financially supported by TNO (Netherlands Organization for Applied Scientific Research), and carried out at TNO innovation for life, Microbiology and Systems biology, Zeist, The Netherlands.

Contents

Outline

Itaconic acid (an unsaturated C5 dicarboxylic acid), as one of the 12 building block chemicals that are the most interesting to be produced by industrial biotechnology, is used in the manufacture of plastics, synthetic fibers, paints etc. To overcome its limited availability at an industrially relevant price from the current production by *Aspergillus terreus*, *Aspergillus niger* is chosen as the production host strain. *A. niger* was selected due to its flexibility as an industrial production platform, and especially due to its accumulation of large amounts of citric acid (360 g/L), which can actually be used as a precursor in the biosynthetic pathway towards itaconic acid. This thesis describes the research carried out for producing itaconic acid in *A. niger*, and illustrates research approaches of organic acids production in fungi in general.

Chapter 1 reviews the industrial production of organic acids especially di, tri-carboxylic acids in fungal species, including the existing or potential metabolic pathways, knowledge on the relevant transporters, production medium and conditions involved in the production process in fungi. *A. niger* is not a natural production host for itaconic acid. In Chapter 2, a clone-based transcriptomics approach was used as a starting step to identify the genes encoding enzymes involved in itaconic acid production from a natural itaconic acid producer, *A. terreus*.

Chapter 3 focused on improving the production level of itaconic acid in *A. niger* by strain engineering and optimizing cultivation media. Two genes – *gpdA* encoding the glyceraldehyde -3-phosphate dehydrogenase (GPD) and *hbd1* encoding a flavohemoglobin domain (HBD) were selected to be overexpressed in *A. niger*. Beyond that, new media were designed based on a reference medium for *A. terreus* and screened using *A. niger* itaconic acid producing strains.

As a follow up from Chapter 2 and 3, Chapter 4 described four aspects to further increase itaconic acid production in *A. niger*: i) expression of two transporters involved in itaconic acid production in *A. terreus*; ii) reduction of oxalic acid by-product formation in the parental strain; iii) use of a host strain with increased citric acid production; iv) modification of oxygen availability in the cultivation process.

In Chapter 5, as a demonstration for the final step of bio-based itaconic acid production, a novel downstream recovery procedure using the native *A. terreus* strain as a study model was explored.