

## University of Groningen

### Thiol-disulfide oxidoreductases in *Bacillus subtilis*

Dorenbos, Ronald

**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:*  
2004

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

*Citation for published version (APA):*

Dorenbos, R. (2004). Thiol-disulfide oxidoreductases in *Bacillus subtilis* Groningen: s.n.

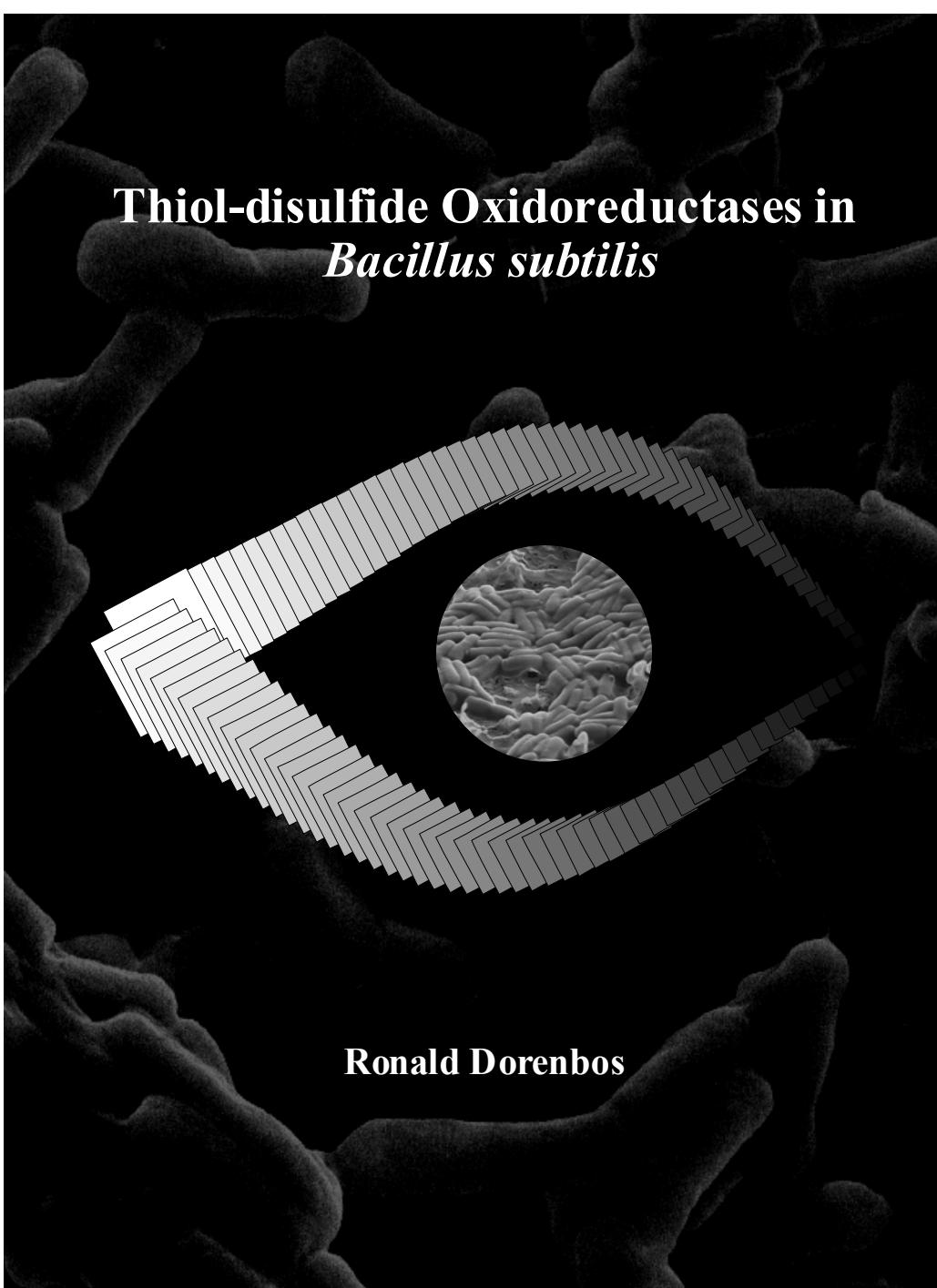
**Copyright**

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).

**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.*



# Thiol-disulfide Oxidoreductases in *Bacillus subtilis*

Ronald Dorenbos



**RIJKSUNIVERSITEIT GRONINGEN**

**Thiol-disulfide Oxidoreductases  
in *Bacillus subtilis***

**Proefschrift**

ter verkrijging van het doctoraat in de  
Wiskunde en Natuurwetenschappen  
aan de Rijksuniversiteit Groningen

op gezag van  
Rector Magnificus, dr. F. Zwarts,  
in het openbaar te verdedigen op  
dinsdag 21 december 2004  
om 13.15 uur

door

**Ronald Dorenbos**

geboren op 8 mei 1972  
te Winschoten

Promotores:  
Prof. Dr. W.J. Quax  
Prof. Dr. J.M. van Dijl

Beoordelingscommissie:  
Prof. Dr. O.P. Kuipers  
Prof. Dr. B. Oudega  
Prof. Dr. M. Sarvas

*Only those who will risk going too far  
can possibly find out how far one can go.*

Thomas Stearns Eliot (1888-1965)

Paranimfen: Joost Folgering

Mattijs Julsing

Voor mijn ouders,

Jaap & Wiesje Dorenbos

This research was performed at the Department of Pharmaceutical Biology of the University of Groningen, the Netherlands. Funding for the work described in this thesis, was provided by the Commission of the European Union Projects BIO4-CT98-0250, QLK3-CT-1999-00413, and QLK3-CT-1999-00917.

Publication of this thesis has been made possible by financial support from the Groningen University Institute for Drug Exploration (GUIDE), Novozymes A/S (Denmark), and Genencor International Inc. (Palo Alto, California). Their contributions are greatly acknowledged.

ISBN: 90-9018901-7

Copyright © 2004 R. Dorenbos. All rights reserved. This book, or any parts thereof, may not be reproduced or transmitted by any means or in any form, without permission from the author.

Cover design by Ronald Dorenbos. Electron microscopic pictures used for the cover, the bookmark, and the chapter heads were made by Ietse Stokroos.





# Contents

|       |  |     |
|-------|--|-----|
| 1     | Introduction Thiol-disulfide Oxidoreductases .....   | 16  |
| 1.1   | Protein production .....   | 16  |
| 1.2   | Thiol-disulfide Oxidoreductases in <i>E. coli</i> .....  | 19  |
| 1.2.1 | DsbA .....   | 21  |
| 1.2.2 | DsbB .....   | 24  |
| 1.2.3 | DsbC .....   | 27  |
| 1.2.4 | DsbD .....   | 29  |
| 1.2.5 | DsbE .....   | 32  |
| 1.2.6 | DsbG .....   | 34  |
| 1.2.7 | Other <i>E. coli</i> proteins implicated in disulfide bond formation.  | 35  |
| 1.3   | Thiol-disulfide Oxidoreductases in <i>B. subtilis</i> .....  | 36  |
| 1.3.1 | <i>Bacillus subtilis</i> .....   | 36  |
| 1.3.2 | The cytoplasm of <i>B. subtilis</i> .....  | 37  |
| 1.3.3 | Identification of thiol-disulfide oxidoreductases in <i>Bacillus</i> ...   | 40  |
| 1.3.4 | BdbA and BdbB .....  | 41  |
| 1.3.5 | BdbC and BdbD .....  | 44  |
| 1.3.6 | Other <i>B. subtilis</i> proteins implicated in disulfide bond formation   | 46  |
| 1.3.7 | Thiol-disulfide Oxidoreductases in Other Organisms .....   | 51  |
| 1.4   | Scope of this thesis .....   | 53  |
| 2     | The <i>bdbDC</i> operon of <i>Bacillus subtilis</i> encodes thiol-disulfide<br>oxidoreductases required for competence development ..... | 56  |
| 2.1   | Summary .....  | 56  |
| 2.2   | Introduction .....   | 57  |
| 2.3   | Experimental Procedures .....  | 59  |
| 2.4   | Results .....  | 64  |
| 2.5   | Discussion .....   | 78  |
| 2.6   | Acknowledgements .....   | 80  |
| 3     | A specific stress response in <i>Bacillus subtilis</i> triggered by secretion of a<br>disulfide bond-containing protein .....            | 82  |
| 3.1   | Summary .....  | 82  |
| 3.2   | Introduction .....   | 83  |
| 3.3   | Experimental procedures .....  | 85  |
| 3.4   | Results .....  | 92  |
| 3.5   | Discussion .....   | 102 |
| 3.6   | Acknowledgements .....   | 107 |
| 4     | Four thiol-disulfide oxidoreductases involved in swarming motility of<br><i>Bacillus subtilis</i> .....                                  | 110 |
| 4.1   | Summary .....  | 110 |
| 4.2   | Introduction .....   | 111 |
| 4.3   | Experimental Procedures .....  | 114 |
| 4.4   | Results .....  | 118 |

|      |  |     |
|------|--|-----|
| 4.5  | Discussion.....  | 123 |
| 4.6  | Acknowledgements .....   | 126 |
| 5    | Thiol-disulfide oxidoreductases are essential for the production of the lantibiotic sublancin 168..... | 128 |
| 5.1  | Summary.....   | 128 |
| 5.2  | Introduction .....   | 129 |
| 5.3  | Experimental procedures .....  | 132 |
| 5.4  | Results .....  | 137 |
| 5.5  | Discussion.....  | 144 |
| 5.6  | Acknowledgements .....   | 148 |
| 6    | YolJ, a CxxS protein required for sublancin 168 production by <i>Bacillus subtilis</i> .....           | 150 |
| 6.1  | Summary.....   | 150 |
| 6.2  | Introduction .....   | 151 |
| 6.3  | Experimental procedures .....  | 153 |
| 6.4  | Results .....  | 160 |
| 6.5  | Discussion.....  | 170 |
| 6.6  | Acknowledgements .....   | 174 |
| 7    | Thiol-disulfide Oxidoreductases of <i>Bacillus subtilis</i> .....                                      | 176 |
| 7.1  | Summary.....   | 176 |
| 7.2  | Introduction .....   | 177 |
| 7.3  | Experimental Procedures .....  | 179 |
| 7.4  | Results .....  | 184 |
| 7.5  | Discussion.....  | 189 |
| 7.6  | Acknowledgements .....   | 192 |
| 8    | Discussion & Future Perspectives .....   | 194 |
| 8.1  | Thiol-disulfide Oxidoreductase-like proteins of <i>B. subtilis</i> .....                               | 194 |
| 8.2  | Thiol-disulfide oxidoreductases and improved production of heterologous proteins.....                  | 199 |
| 8.3  | Wider application potential of thiol-disulfide oxidoreductases .....                                   | 202 |
| 8.4  | Pathogenicity and thiol-disulfide oxidoreductases .....  | 204 |
| 8.5  | Concluding Remarks .....   | 207 |
| 9    | Reference List.....  | 210 |
| 10   | Summary & Samenvatting.....  | 234 |
| 10.1 | Summary.....   | 234 |
| 10.2 | Samenvatting .....   | 238 |
| 11   | Dankwoord .....  | 244 |
| 12   | Curriculum Vitae & Publications .....  | 254 |
| 12.1 | Curriculum Vitae .....   | 254 |
| 12.2 | Publications .....   | 255 |



