University of Groningen

From the wound to the bench

García Pérez, Andrea

DOI:
[10.33612/diss.128078435](https://doi.org/10.33612/diss.128078435)

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:
2020

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

Citation for published version (APA):

García Pérez, A. (2020). *From the wound to the bench: A study of wound-colonising bacteria and their interactions*. [Thesis fully internal (DIV), University of Groningen]. University of Groningen.
<https://doi.org/10.33612/diss.128078435>

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.

**From the wound to the bench:
A study of wound-colonising bacteria
and their interactions**

Andrea N. García-Pérez

The work described in this thesis was performed in the Laboratory of Molecular Bacteriology, Department of Medical Microbiology of the University of Groningen and University Medical Center Groningen (The Netherlands).

This PhD research was financially supported by the Graduate School of Medical Sciences (GSMS) of the University of Groningen, CoNaCyT, the Eleven Floowers Fund, and the generous sponsors of the Ubbo Emmius Fund's crowdfunding project 'Beat the bacterium'.

From the wound to the bench: A study of wound-colonising bacteria and their interactions
Dissertation of the University of Groningen

ISBN: 978-94-034-2799-7 (printed version)

ISBN: 978-94-034-2800-0 (electronic version)

Copyright © 2020 Andrea N. García-Pérez

Book cover: Electron Microscope Unit at the University of Cape Town

Supplemental files: <https://unishare.nl/index.php/s/DERoqdHLazYZ4Q9>

The printing of this thesis was financially supported by the Graduate School of Drug Exploration (GUIDE) and the University of Groningen. Their support is highly appreciated.

All rights reserved. No parts of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, without the written permission of the author. The copyright of previously published chapters of this thesis also remains with the publisher or journal.

Printed by Gildeprint Drukkerijen, Enschede, the Netherlands

university of
 groningen

From the wound to the bench

A study of wound-colonising bacteria and their
 interactions

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

Wednesday 1 July 2020 at 16.15 hours

by

Andrea Nayelli García Pérez

born on 19 July 1982
 in Mexico City, Mexico

Supervisors:

Prof. J. M. van Dijk

Dr. G. Buist

Assessment Committee:

Prof. W. J. Quax

Prof. B. C. Kahl

Prof. J. Kok

Paranymphs:

Rocío Aguilar-Suárez

Marines du Teil

Adriana Pérez Fortis

A mi madre, mi padre y 'Saroo'

Contents

1. General introduction and scope of the thesis	1
1.1. Introduction	2
1.2. Scope and outline of this thesis	5
2. Epideromyolysis bullosa subtypes and the wound microbiome: a pilot study	11
2.1. Introduction	12
2.2. Materials and Methods	13
2.2.1. Ethics statement	13
2.2.2. Study participants and sample collection	15
2.2.3. DNA extraction	16
2.2.4. Choice of universal primers	17
2.2.5. PCR amplification	18
2.2.6. 16S rRNA gene sequencing and analysis	18
2.3. Results	19
2.3.1. Phenol-chloroform based method resulted in better DNA yields	19
2.3.2. Differences among chronic and acute wounds were observed in several taxonomic levels	19
2.3.3. <i>S. aureus</i> was abundant in both acute and chronic wounds . .	22

2.3.4.	Different wound microbiomes among EB subtypes	25
2.4.	Discussion	26
3.	<i>S. aureus</i> exoproteomes in co-culture	31
3.1.	Introduction	32
3.2.	Results	34
3.2.1.	<i>S. aureus</i> grows unimpaired when co-cultured with <i>B. thuringien-</i> <i>sis</i> or <i>K. oxytoca</i> on solid agar	34
3.2.2.	<i>S. aureus</i> isolates t111 and t13595 are highly related but not identical	35
3.2.3.	Total numbers of <i>S. aureus</i> exoproteins are reduced when <i>S. aureus</i> is co-cultured with Bt or Ko	37
3.2.4.	Co-cultured <i>S. aureus</i> , <i>B. thuringiensis</i> and <i>K. oxytoca</i> isolates display species-specific exoproteome changes in terms of biological processes	40
3.2.5.	Particular exoproteins of <i>S. aureus</i> , <i>K. oxytoca</i> and <i>B. thuringien-</i> <i>sis</i> show quantitative changes in mono- or co-cultures	42
3.3.	Discussion	48
3.4.	Material and Methods	52
3.4.1.	Ethical approval	52
3.4.2.	Bacterial isolation, identification and culture conditions	52
3.4.3.	Genomic DNA extraction, genome sequencing and analysis	53
3.4.4.	Preparation of protein extracts	54
3.4.5.	Mass Spectrometry and data analysis	55
3.4.6.	Bioinformatic analyses	56

3.4.7.	Biological and Chemical Safety	56
3.4.8.	Ethics	57
3.4.9.	Funding	57
3.4.10.	Disclosure of Potential Conflicts of Interest	57
3.4.11.	Acknowledgements	57
4.	Staphylococcal interactions with wound-colonising bacteria	69
4.1.	Introduction	70
4.2.	Methods	72
4.2.1.	Strains and growth conditions	72
4.2.2.	Preparation of protein extracts	72
4.2.3.	Mass spectrometry analyses	74
4.2.4.	RNA isolation	74
4.2.5.	RNA sequencing and analysis	75
4.2.6.	<i>Galleria mellonella</i> infections	77
4.2.7.	Data Summary	78
4.3.	Results	78
4.3.1.	Profiling of the <i>S. aureus</i> cellular proteome reflects decreased virulence upon culturing with <i>K. oxytoca</i>	78
4.3.2.	Co-culturing leads to upregulation of <i>S. aureus</i> genes involved in amino acid and nucleotide metabolism and transport under the tested conditions	83
4.3.3.	Planktonic <i>S. aureus</i> displays specific gene expression signatures upon co-culturing	85

4.3.4.	<i>S. aureus</i> shows massive silencing of gene expression upon co-culturing under biofilm-mimicking conditions	86
4.3.5.	Reduced mortality of <i>Galleria mellonella</i> upon co-infection with <i>S. aureus</i> and <i>K. oxytoca</i>	88
4.4.	Discussion	89
4.5.	Author Statements	97
4.5.1.	Author contributions	97
4.5.2.	Conflicts of interest	97
4.5.3.	Funding	97
4.5.4.	Acknowledgements	97
5.	Metatranscriptomic snapshot of <i>S. aureus</i> and <i>C. striatum</i>	105
5.1.	Introduction	106
5.2.	Materials and methods	107
5.2.1.	Ethics Statement	107
5.2.2.	Wound sampling and RNA extraction	108
5.2.3.	16S rRNA gene sequencing and analysis	108
5.2.4.	RNA sequencing and metatranscriptome analysis	109
5.3.	Results and discussion	111
5.4.	Data availability	116
5.5.	Conflict of interest	116
5.6.	Acknowledgements	117
5.7.	Author contributions	117

5.8. Funding	118
6. Summary and future perspectives	121
Appendices	129
Samenvatting en perspectief voor toekomstig onderzoek	130
Resumen y perspectivas a futuro	139
Acknowledgements	147

List of Figures

1.1	Thin human skin section	4
1.2	Schematic representation of the distinct steps in microbial biofilm development	5
1.3	Schematic diagram of different characteristics of bacterial biofilm .	6
2.1	Agarose gel for detection of amplified bacterial 16S rRNA gene regions V1-V3	21
2.2	Species diversity	23
2.3	Most abundant bacterial species	24
2.4	Principal component analysis of the microbiome composition based on the classification table at the genus level	25
2.5	Most abundant bacterial species identified in wound samples from patients with different EB subtypes	27
3.1	Growth characteristics of the isolated bacteria on agar plates	36
3.2	Total numbers of extracellular proteins identified per culture	39
3.3	Relationships among cultures	41
3.4	Overview of the predicted functions of the exoproteins found in all staphylococcal cultures	43
4.1	Experimental workflow	79
4.2	Cellular proteins identified per culture	80

4.3	Visualization of global predicted functions of cellular proteins identified in all staphylococcal cultures (stationary phase)	81
4.4	Overview of co-culture experiments according to Clusters of Orthologous Groups (COG)	84
4.5	Differentially expressed genes (DEG) of <i>S. aureus</i> upon co-culturing in liquid and on agar media	87
4.6	Mortality of <i>Galleria mellonella</i> larvae upon (co)infection with <i>S. aureus</i> t111, t13595, <i>B. thuringiensis</i> and <i>K. oxytoca</i>	90
5.1	Functional classification of the staphylococcal metatranscriptome .	113

List of Tables

2.1	Classification and genetic abnormalities of Epidermolysis Bullosa (EB)	14
2.2	Sample registration from swabs (SWB) or bandages (BDG)	15
2.3	DNA concentrations with different extraction methods.	20
3.1	Summary of differentially detected extracellular proteins among the different cultures	44
4.1	Functional analysis of the different data sets (mono or co-culture) from <i>S. aureus</i> t111, t13595, <i>B. thuringiensis</i> and <i>K. oxytoca</i> during exponential phase	92
4.2	Functional analysis of the different data sets (mono or co-culture) from <i>S. aureus</i> t111, t13595, <i>B. thuringiensis</i> and <i>K. oxytoca</i> during stationary phase	93
5.1	Overview of genes associated to virulence, disease and defence expressed <i>in situ</i> vs. co-culture.	114
5.2	Overview of genes associated to virulence, disease and defence expressed <i>in situ</i> vs monoculture.	115