

Table of Contents

1	Genome and proteome analysis of <i>Chlamydia</i>	1
	<i>Brian B. S. Vandahl, Svend Birkelund and Gunna Christiansen</i>	
1.1	Introduction	1
1.1.1	<i>Chlamydia</i> biology	2
1.1.1.1	Diseases	2
1.1.1.2	The developmental cycle	3
1.2	<i>Chlamydia</i> genomes	5
1.2.1	Sequenced <i>Chlamydia</i> genomes	5
1.2.2	<i>Chlamydia</i> genes	6
1.2.3	Genome comparison	7
1.3	Proteome analysis of <i>Chlamydia</i>	9
1.3.1	Early <i>Chlamydia</i> proteome studies	10
1.3.2	<i>C. trachomatis</i> proteome studies	10
1.3.3	<i>C. pneumoniae</i> proteome studies	11
1.3.4	Identification of secreted proteins by comparative proteomics	13
1.3.5	Proteome studies of comc	15
1.3.6	Proteome comparison of <i>S. trachomatis</i> serovars	15
1.3.7	Proteome analysis of growth conditions	16
1.3.8	Considerations in proteomics	16
1.4	Concluding remarks	17
2	<i>Helicobacter pylori</i> vaccine development based on combined subproteome analysis	21
	<i>Dirk Bumann, Peter R. Jungblut and Thomas F. Meyer</i>	
2.1	Introduction	21
2.2	Classical whole-cell inactivated <i>Helicobacter</i> vaccines	22
2.3	Subunit <i>Helicobacter</i> vaccines: Conventional antigen selection	22
2.4	Subunit <i>Helicobacter</i> vaccines: Global antigen selection based on proteomics	23
2.4.1	Proteomics as a tool for antigen characterization	23

2.4.2	The <i>Helicobacter</i> proteome	24
2.4.3	Criteria for promising antigen candidates	25
2.4.4	Identification of protective antigens based on multiple criteria	27
2.5	Concluding remarks	28
3	Towards a comprehensive understanding of <i>Bacillus subtilis</i> cell physiology by physiological proteomics	31
	<i>Michael Hecker and Uwe Völker</i>	
3.1	Introduction	32
3.2	Subproteomes vs. the total theoretical proteome	33
3.3	The vegetative proteome of growing cells	34
3.4	Proteomes of nongrowing cells – the adaptational network	39
3.5	Proteomic signatures – tools for microbial physiology and their practical application	48
3.6	Transcriptomics vs. proteomics – towards a second generation of proteomics	50
3.7	The interactome	52
3.8	The secretome	53
3.9	Post-translational modifications	53
3.10	Protein quality control/protein degradation at a proteomic scale	55
3.11	Gene expression network – horizontal and vertical approach	56
3.12	Concluding remarks	58
4	Web-accessible proteome databases for microbial research	63
	<i>Klaus-Peter Pleißner, Till Eifert, Sven Buettner, Frank Schmidt, Martina Boehme, Thomas F. Meyer, Stefan H. E. Kaufmann and Peter R. Jungblut</i>	
4.1	Introduction	64
4.2	Materials and methods	64
4.2.1	Data generation and data storage	64
4.2.2	Software tools	65
4.3	Results and discussion	65
4.3.1	Data management, analysis and presentation	65
4.3.2	2D-PAGE database	66
4.3.3	ICAT-LC/MS database	68
4.3.4	FUNC_CLASS database	68
4.3.5	Data analysis and visualization	69
4.4	Concluding remarks	73

5 A targeted proteomics approach to the rapid identification of bacterial cell mixtures by matrix-assisted laser desorption/ionization mass spectrometry 75

Bettina Warscheid and Catherine Fenselau

- 5.1 Introduction 75
- 5.2 Materials and methods 76
 - 5.2.1 Chemicals 76
 - 5.2.2 *Bacillus* strains 77
 - 5.2.3 Vegetative cell digestion 77
 - 5.2.4 MALDI-TOF MS and unimolecular decomposition product ion analysis 77
 - 5.2.5 Database searches and identification of *Bacillus* species 78
- 5.3 Results and discussion 79
 - 5.3.1 On-probe tryptic digestion of bacterial cells 79
 - 5.3.1.1 *Bacillus subtilis* 168 79
 - 5.3.1.2 *Bacillus globigii* and *sphaericus* 14577 79
 - 5.3.1.3 *Bacillus cereus* T and *anthracis* Sterne 81
 - 5.3.2 Partial sequencing for rapid identification of bacterial cells 83
 - 5.3.2.1 Unimolecular decomposition product ion analysis (UDPIA) 83
 - 5.3.2.2 Identification of bacteria 85
 - 5.3.3 Identification of proteins and protein-families in bacterial cell digests 85
 - 5.3.3.1 Flagellin and surface layer protein precursor 85
 - 5.3.3.2 Cold shock and cold shock-like proteins 87
 - 5.3.3.3 Ribosomal proteins 90
 - 5.3.3.4 DNA-binding proteins 90
 - 5.3.3.5 Heat shock proteins 91
 - 5.3.3.6 Other stress related proteins and the prosthetic group of an acyl-carrier protein 92
 - 5.3.4 Analysis of a 1:1 mixture of *B. globigii* and *B. sphaericus* 14577 92
- 5.4 Concluding remarks 94

6 Protein identification and tracking in two-dimensional electrophoretic gels by minimal protein identifiers 97

Jens Mattow, Frank Schmidt, Wolfgang Höhenwarter, Frank Siejak, Ulrich E. Schaible and Stefan H. E. Kaufmann

- 6.1 Introduction 98
- 6.2 Materials and methods 99
 - 6.2.1 Materials 99
 - 6.2.2 Two-dimensional electrophoresis 100
 - 6.2.3 Mass spectrometry 100

6.2.4	Compilation of a theoretical dataset comprising all tryptic peptides of <i>M. tuberculosis</i> H37Rv	100
6.2.5	Comparison of MALDI spectra by the program MS-Screener	101
6.2.6	Determination of exogenous contaminant masses	101
6.2.7	Generation of template spectra	102
6.3	Results and discussion	102
6.3.1	Proteome analysis of <i>M. tuberculosis</i> H37Rv CSN	102
6.3.2	Determination of exogenous contaminant masses by MS-Screener	102
6.3.3	The MPI approach revealed HspX-specific peptide masses in multiple spectra	106
6.3.4	The MS-Screener analysis revealed truncated variants of Tuf previously not identified by PMF	111
6.3.5	Frequency of tryptic peptides with similar m/z ratios in <i>M. tuberculosis</i> H37Rv	113
6.3.6	In mass spectrometry it is important to consider detection probabilities of proteins and peptides	114
6.4	Concluding remarks	117
7	Continued proteomic analysis of <i>Mycobacterium leprae</i> subcellular fractions	121
	<i>Maria Angela M. Marques, Benjamin J. Espinosa, Erika K. Xavier da Silveira, Maria Cristina V. Pessolani, Alex Chapeaurouge, Jonas Perales, Karen M. Dobos, John T. Belisle, John S. Spencer and Patrick J. Brennan</i>	
7.1	Introduction	121
7.2	Materials and methods	125
7.2.1	Preparation and separation of <i>M. leprae</i> proteins	125
7.2.2	MS	126
7.2.3	Isolation of basic proteins of <i>M. leprae</i>	127
7.3	Results and discussion	127
7.4	Concluding remarks	138
8	CFP10 discriminates between nonacetylated and acetylated ESAT-6 of <i>Mycobacterium tuberculosis</i> by differential interaction	141
	<i>Limei Meng Okkels, Eva-Christina Müller, Monika Schmid, Ida Rosenkrands, Stefan H. E. Kaufmann, Peter Andersen and Peter R. Jungblut</i>	
8.1	Introduction	141
8.2	Materials and methods	142
8.2.1	Protein samples	142
8.2.2	2-DE blot overlay	143
8.2.3	Mass spectrometry	143
8.3	Results	144
8.3.1	High resolution separation of acidic ST-CF proteins	144

8.3.2	Mass analysis of ESAT-6 spots	144
8.3.3	Interaction of recombinant CFP10 with ESAT-6 spots	149
8.4	Discussion	150
9	The cell wall subproteome of <i>Listeria monocytogenes</i>	153
	<i>Jessica Schaumburg, Oliver Diekmann, Petra Hagendorff, Simone Bergmann, Manfred Rohde, Sven Hammerschmidt, Lothar Jänsch, Jürgen Wehland, and Uwe Käst</i>	
9.1	Introduction	153
9.2	Material and methods	154
9.2.1	Bacterial strain and growth conditions	154
9.2.2	Serial extraction of cell wall proteins	155
9.2.3	Aminopeptidase C assay	155
9.2.4	SDS-PAGE Western blotting, and N-terminal sequencing	155
9.2.5	2-D-PAGE	155
9.2.6	Gel staining and protein identification by mass spectrometry	156
9.2.7	Immunoelectron microscopy	156
9.2.7.1	Postembedding labeling studies	156
9.2.7.2	Field emission scanning electron microscopic immunolabeling	157
9.2.8	Overlay blot	157
9.2.9	Ligand fishing	158
9.2.10	Cloning and purification of proteins	158
9.2.11	Kinetic analyses using SPR detection	159
9.2.12	Bioinformatic analysis	159
9.3	Results	160
9.3.1	Prediction and validation of cell wall-associated proteins	160
9.3.1.1	Prediction of exported proteins	160
9.3.1.2	Validation of the cell wall subproteome	160
9.3.2	Analysis of protein processing and localization	167
9.3.3	Analysis of plasminogen-binding proteins	168
9.4	Discussion	173
9.4.1	Analysis of proteins identified from surface extracts	173
9.4.2	Analysis of protein processing and localization in surface extracts	174
9.4.3	Possible function of plasminogen binding in virulence	175
9.5	Concluding remarks	177
10	Low virulent strains of <i>Candida albicans</i>: Unravelling the antigens for a future vaccine	181
	<i>Elena Fernández-Arenas, Gloria Molero, César Nombela, Rosalía Díez-Orejas and Concha Gil</i>	
10.1	Introduction	182
10.2	Materials and methods	183

10.2.1	Microorganism and culture conditions	183
10.2.2	Mice	183
10.2.3	Systemic infection conditions and generation of immune sera	183
10.2.4	T cell purification and passive immunization	184
10.2.5	2-DE	184
10.2.5.1	Protoplast lysate preparation	184
10.2.5.2	Analytical and micropreparative 2-DE	185
10.2.6	Immunoblot analyses	185
10.2.7	MALDI-TOF and MALDI-TOF MS analyses of spots	186
10.2.8	Database search	187
10.3	Results	187
10.3.1	Vaccination assays with different mutant strains and generation of immune sera	187
10.3.2	Importance of cellular immunity in vaccination with <i>C. albicans</i> CNC13	188
10.3.2.1	Role of Th1/Th2 cytokines in CNC13 immune response	188
10.3.2.2	Protection induced by passive transfer of sensitized CNC13 lymphocytes	189
10.3.3	Profile of <i>C. albicans</i> immunoreactive proteins in the different mutant strains	190
10.3.3.1	Detection and identification of the immunoreactive proteins	190
10.4	Discussion	194
10.4.1	Low virulent <i>C. albicans</i> strains as a tool to study the host immune response	194
10.4.2	<i>C. albicans</i> <i>hog1</i> mutant induces protection in a vaccination assay	196
10.4.3	<i>C. albicans</i> new antigenic proteins	197
10.4.4	Antibody profile linked to successful vaccination against systemic candidiasis	197
10.5	Concluding remarks	198
11	Proteomic analysis of the sarcosine-insoluble outer membrane fraction of the bacterial pathogen <i>Bartonella henselae</i>	203
	Thomas A. Rhomberg, Olof Karlberg, Thierry Mini, Ursula Zimny-Arndt, Ulrika Wickenberg, Marlene Röttgen, Peter R. Jungblut, Paul Jenö, Siv G. E. Andersson and Christoph Dehio	
11.1	Introduction	203
11.2	Materials and methods	205
11.2.1	Strains and culture conditions	205
11.2.2	Enrichment of <i>B. henselae</i> OMPs	205
11.2.3	Protease exposure	205
11.2.4	1-D SDS-PAGE	206

11.2.5	Protein solubilization and protein quantitation	206
11.2.6	2-D NEPHGE	206
11.2.7	MALDI-TOF-MS	207
11.2.8	Database query	208
11.2.9	<i>In silico</i> analysis	208
11.3	Results	208
11.3.1	Enrichment of <i>B. henselae</i> OMPs	208
11.3.2	1-D SDS-PAGE of <i>B. henselae</i> OMPs and protein assignment by PMF	209
11.3.3	2-D NEPHGE of <i>B. henselae</i> OMPs and protein assignment by PMF	211
11.4	Discussion	215
12	The influence of <i>agr</i> and σ^B in growth phase dependent regulation of virulence factors in <i>Staphylococcus aureus</i>	225
	<i>Anne-Kathrin Ziebandt, Dörte Becher, Knut Ohlsen, Jörg Hacker, Michael Hecker and Susanne Engelmann</i>	
12.1	Introduction	225
12.2	Material and methods	227
12.2.1	Bacterial strains and culture conditions	227
12.2.2	Preparation of the extracellular protein fraction	227
12.2.3	Analytical and preparative PAGE	227
12.2.4	Quantitation of protein spots	228
12.2.5	Transcriptional analyses	228
12.3	Results	228
12.3.1	Growth phase dependent regulation of extracellular proteins	228
12.3.2	The influence of <i>agr</i> and σ^B on the extracellular proteome	236
12.3.3	The influence of <i>agr</i> and σ^B on the transcription of virulence genes	239
12.3.4	Effect of σ^B on the transcription of <i>agr</i> and <i>sarA</i>	239
12.4	Discussion	239
12.5	Concluding remarks	245
13	Comparative proteome analysis of cellular proteins extracted from highly virulent <i>Francisella tularensis</i> ssp. <i>tularensis</i> and less virulent <i>F. tularensis</i> ssp. <i>holarctica</i> and <i>F. tularensis</i> ssp. <i>mediaasiatica</i>	249
	<i>Martin Hubálek, Lenka Hernychová, Martin Brychta, Juraj Lenčo, Jana Zechovská and Jiří Stulík</i>	
13.1	Introduction	250
13.2	Material and methods	251
13.2.1	Bacterial cultures and sample preparation for 2-DE	251
13.2.2	Two-dimensional gel electrophoresis	252
13.2.3	Statistical analysis	252

13.2.4	In-gel digestion	253
13.2.5	Mass spectrometric identification	253
13.3	Results and discussion	254
13.4	Concluding remarks	265
14	Proteome comparison of <i>Vibrio cholerae</i> cultured in aerobic and anaerobic conditions	267
	<i>Biao Kan, Hajar Habibi, Monika Schmid, Weili Liang, Ruibai Wang, Duochun Wang and Peter R. Jungblut</i>	
14.1	Introduction	267
14.2	Materials and methods	269
14.2.1	Strains and culture	269
14.2.2	Sample preparation for 2-DE	269
14.2.3	2-DE	269
14.2.4	MS	270
14.2.5	Electron microscope	270
14.3	Results and discussion	270
14.3.1	Identified protein species more abundant in aerobic culture	274
14.3.2	Identified protein species which are more abundant in anaerobic culture	275
14.4	Concluding remarks	276
15	Induction of <i>Mycobacterium avium</i> proteins upon infection of human macrophages	279
	<i>Lara Brunori, Federico Giannoni, Luca Bini, Sabrina Liberatori, Cristiane Frota, Peter Jenner, Ove Fredrik Thoresen, Graziella Orefici and Lanfranco Fattorini</i>	
16	Proteomics-based identification of novel <i>Candida albicans</i> antigens for diagnosis of systemic candidiasis in patients with underlying hematological malignancies	289
	<i>Aida Pitarch, Joaquín Abian, Montserrat Carrascal, Miguel Sánchez, César Nombela and Concha Gil</i>	
16.1	Introduction	290
16.2	Materials and methods	291
16.2.1	Human serum samples	291
16.2.2	Preparation of <i>C. albicans</i> protoplast lysates	292
16.2.3	Two-dimensional polyacrylamide gel electrophoresis (2-D PAGE)	292
16.2.4	Immunoblot analysis	294
16.2.5	Peptide sample preparation for MS and MS/MS analyses	294
16.2.5.1	In-gel digestion	294
16.2.5.2	Desalting	294

16.2.6	Mass spectrometric analysis (MS and MS/MS)	295
16.2.6.1	Peptide mapping by MALDI-TOF-MS	295
16.2.6.2	Peptide fragmentation and sequencing by nanoESI-IT-MS	295
16.2.7	Database searching	296
16.3	Results	296
16.3.1	Mapping of <i>C. albicans</i> immunogenic proteins	296
16.3.1.1	Overall 2-D <i>C. albicans</i> antigen recognition pattern	296
16.3.1.2	Identification of <i>C. albicans</i> immunoreactive proteins by peptide mass fingerprinting (PMF)	297
16.3.1.3	Peptide sequencing of <i>C. albicans</i> immunoreactive proteins	298
16.3.1.4	Reference 2-DE <i>C. albicans</i> antigen pattern display on the Net	305
16.3.2	Comparison of 2-D <i>C. albicans</i> antigen recognition patterns obtained with serum samples from patients with and without systemic candidiasis	309
16.3.3	Differences in the 2-D <i>C. albicans</i> antigen recognition profile associated with infection progression	311
16.4	Discussion	312
16.4.1	<i>C. albicans</i> housekeeping enzymes can stimulate the human immune system during systemic candidiasis	316
16.4.1.1	Heat shock proteins (Hsps)	317
16.4.1.2	Metabolic proteins	318
16.4.1.3	Elongation factors and ribosomal proteins	318
16.4.1.4	Miscellaneous proteins	319
16.4.2	Natural anti- <i>Candida</i> antibodies might be correlated with differentiation of the human immune response	319
16.4.3	Serum levels of specific anti- <i>Candida</i> antibodies could be useful for the clinical follow-up of systemic candidiasis	320
	Index	325