DEVELOPMENT AND EVALUATION OF MUCOSAL TUBERCULOSIS VACCINE UTILIZING IN-TRANS SURFACE DISPLAY SYSTEM OF Lactobacillus plantarum Pa21 IN MICE

ANHAR DANIAL BIN MUSTAFA

FBSB 2017 50

DEVELOPMENT AND EVALUATION OF MUCOSAL TUBERCULOSIS VACCINE UTILIZING IN-TRANS SURFACE DISPLAY SYSTEM OF *Lactobacillus plantarum* Pa21 IN MICE

By

ANHAR DANIAL BIN MUSTAFA

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

November 2017

© COPYRIGHT UPM

All material contained within the thesis, including without limitation text, logos, icons, photographs and all other artwork, is copyright material of Universiti Putra Malaysia unless otherwise stated. Use may be made of any material contained within the thesis for non-commercial purposes from the copyright holder. Commercial use of material may only be made with the express, prior, written permission of Universiti Putra Malaysia.

Copyright © Universiti Putra Malaysia

© COPYRIGHT UPM

This thesis is dedicated to my wife and children.

© COPYRIGHT UPM

Abstract of a thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

DEVELOPMENT AND EVALUATION OF MUCOSAL TUBERCULOSIS VACCINE UTILIZING *IN-TRANS* SURFACE DISPLAY SYSTEM OF *Lactobacillus plantarum* Pa21 IN MICE

By

ANHAR DANIAL BIN MUSTAFA

November 2017

Chairperson: Professor Khatijah Yusoff, PhD
Faculty : Biotechnology and Bimolecular Science

The severity of tuberculosis is significant since it has been estimated to have latently affected almost a third of the world's population with *Mycobacterium tuberculosis*, the causative agent for tuberculosis. Fortunately, the disease is being prevented and controlled by the Bacille Calmette Guérin (BCG) vaccine, the only viable vaccine approved for human usage since 1921. Nevertheless, the protective efficacy of the BCG vaccine has been highly variable and substandard (0-80%) in adults especially in endemic regions due to pre-existing immune responses to the vaccine. Therefore, there is a strong need for new and effective tuberculosis vaccine to be developed either as a replacement or as a booster in order to overcome the limitations from BCG vaccine. In this project, candidate mucosal tuberculosis vaccines utilizing protein surface display system of *Lactobacillus plantarum* carrying and displaying five combinations of *M. tuberculosis* subunit epitope antigens (Ag85B, CFP-10, ESAT-6, Rv0475 and Rv2031c) fused with LysM anchor motif were constructed, cloned and expressed in *Esherichia coli* Rossetta expression host. By using the *in trans* approach, the recombinant protein of interest were expressed in *E. coli* and extracted before being applied for extracellular attachment onto the cell wall of *L. plantarum*. Subsequently the binding capability of the recombinant protein of interest to the cell wall of *L. plantarum* was examined via the immunofluorescence microscopy and whole cell ELISA approaches where successful attachment and consistent stability of cell wall binding up to four days period was determined. Although the five recombinant protein of interest were expressed successfully, their expressions were found to be deposited in the inclusion bodies (IBs) of the *E. coli*. To overcome this, the solubilization on the IBs was optimized via the 5% (w/v) N-lauryl sarcosine treatment but among the five recombinant protein of interest, only ACERL was managed to be recovered optimally (38.2% extractability) for further usage in animal study. Therefore the immunization of the female Balb/c mice with the developed vaccine of *L. plantarum* surface displaying ACERL (Lp ACERL) via the oral and intranasal route of administration were studied for its immunogenicity effects. Significant increase in total IgG antibody response and high CD4+/CD8+ ratio from the Lp ACERL immunized oral group as compared to nasal group indicates high reactivity

© COPYRIGHT UPM

of the developed vaccine in being recognized by the host immune system. Moreover, unlike the nasal group, Lp ACERL of orally immunized mice was able to invoke strong mucosal and systemic immune responses as indicated by the specific IgA antibody response from the immunized mice's gastrointestinal tract (GIT), feacal and serum samples. More importantly, Lp ACERL of orally immunized mice showed immune response that favors the Th1 type response as indicated by the immune response outcome from the cytokine profiling of spleen, lung and GIT, and the re-stimulation of the splenocytes from the immunized mice. In contrast, the Lp ACERL intranasally immunized mice does not show significant correlation for inducing neither Th1 nor Th2 immune response. However, this may be due to severe inflammation on the lung of the nasally immunized mice that might compromised its overall immune response outcome. The effect of adding adjuvant consisting of *Lactococcus lactis* secreting mouse IL-12 (LcIL-12) co-administered with Lp ACERL was also determined as it shown to induced a more favorable condition for the candidate vaccine to further generate significant and bias of Th1 protective immune responses as compared to the immune response from the immunization with Lp ACERL alone. The enhancement of the Th1 type responses were observed from the serum IgG sub-types and the cytokine profiling of spleen, lung and GIT immune response outcome. Thus, this study shows that the developed candidate vaccine of Lp ACERL with or without LcIL-12 adjuvant was able to stimulate favorable Th1 type response which is the key indicator for inducing effective protective memory response required for the prevention of tuberculosis. In conclusion,, this study represents a proof of concept in the development of *L. plantarum* as a carrier for a non-genetically modified organism (GMO) tuberculosis vaccine, which may be the strategy in the future for tuberculosis vaccine development.

© COPYRIGHT UPM

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PEMBANGUNAN DAN PENILAIAN VAKSIN TUBERKULOSIS MUKOSA MENGGUNAKAN SISTEM PAPARAN PERMUKAAN *Lactobacillus plantarum* Pa21 SECARA *IN-TRANS* TERHADAP TIKUS

Oleh

ANHAR DANIAL BIN MUSTAFA

November 2017

Pengerusi : Professor Khatijah Yusoff, PhD
Fakulti : Bioteknologi dan Sains Biomolekul

Keterukan kesan daripada tuberkulosis adalah ssangat ketara kerana ianya telah dianggarkan hampir satu pertiga daripada penduduk dunia sudah secara terpendam dijangkiti *Mycobacterium tuberculosis*, iaitu agen penyebab untuk tuberkulosis. Mujur, penyakit ini dapat dikawal serta dicegah oleh Bacille Calmette Guerin (BCG) vaksin, satu-satunya vaksin yang diluluskan untuk penggunaan manusia sejak tahun 1921. Walau bagaimanapun, keberkesanan perlindungan vaksin BCG adalah berbeza-beza dan subpiawai (0-80%) terutamanya tindak balas imun terhadap vaksin di kawasan-kawasan endemik yang telah sedia terkesan daripada tindak balas vaksin tersebut. Oleh itu, terdapat keperluan yang kukuh untuk vaksin tuberkulosis yang baru dan berkesan untuk dibangunkan sama ada sebagai pengganti atau penggalak untuk mengatasi kelemahan daripada vaksin BCG. Dalam projek ini, calon untuk vaksin mukosa tuberkulosis menggunakan sistem paparan permukaan protein *Lactobacillus plantarum* membawa dan memaparkan lima kombinasi *M. tuberculosis* antigen epitop subunit (Ag85B, CFP-10, ESAT-6, Rv0475 dan Rv2031c) bergabung dengan LysM sebagai motif pautan di mana pembinaan, pengklonan dan ekspresi berlaku di dalam perumah ekspresi *Esherichia coli* Rosetta. Dengan menggunakan pendekatan *in-trans*, pengeskpresan and ekstrak protein rekombinan berkepentingan dalam *E. coli* dilakukan dahulu sebelum dilampirkan secara luar sel ke dinding sel *L. plantarum*. Selepas itu keupayaan pautan protein rekombinan berkepentingan ke sel dinding *L. plantarum* telah diperiksa melalui mikroskop imunopendarfluor dan ELISA sel menyeluruh di mana pautan yang berjaya dan kestabilan yang konsisten sehingga tempoh empat hari telah ditentukan.Walaupun lima protein rekombinan berkepentingan telah berjaya diekspresikan, ekspresi mereka didapati tersimpan di jasad rangkuman (IB) di dalam *E. coli* berkenaan. Bagi mengatasinya, perlarutan pada IBs telah dioptimumkan melalui 5% (w/v) rawatan N-lauryl sarcosine akan tetapi di antara lima protein rekombinan berkepentingan, hanya ACERL telah berjaya dipulihkan secara optimum (38.2% keterekstrakan) untuk kegunaan seterusnya dalam kajian haiwan. Oleh itu, imunisasi daripada tikus mincit betina BALB/c dengan vaksin yang di bina daripada *L. plantarum* yang permukaan sel dindingnya memaparkan ACERL (Lp ACERL) melalui laluan mulut dan intranasal telah

© COPYRIGHT UPM

dikaji bagi melihat kesan keimunogenan. Peningkatan ketara IgG antibodi dan ratio CD4+.CD8+ daripada kumpulan tikus mincit yang telah diimunisasi dengan Lp ACERL berbanding kumpulan nasal telah menunjukkan peningkatan reaktiviti oleh vaksin yang dibangunkan terhadap pengenalan sistem imunisasi induk. Seterusnya, berbanding kumpulan nasal, tikus mincit yang telah diimunisasi dengan Lp ACERL secara mulut dapat memberikan gerak balas imun yang kuat terhadap sistem imunisasi daripada mukosa dan gerak balas sistemik berdasarkan tindak-balas spesifik IgA antibodi daropada salur gastrousus, najis dan serum sampel oleh tikus mincit yang telah diimmunisasi. Seterusnya, tikus mincit yang telah diimunisasi oleh Lp ACERL telah menunjukkan kesan immunisasi yang menjurus kepada jenis Th1 respon yang berdasarkan kesan immunisasi terhadap profil cytokine daripada limpa, paru-paru dan salur gastrousus serta stimulasi semula oleh splenocytes daripada tikus mincit yang telah diimunisasi. Sebaliknya, tikus mincit imunisasi dengan Lp ACERL secara intranasal tidak menunjukkan hubungan yang signifikan untuk mendorong tidak tindak balas imun Th1 mahupun Th2. Walau bagaimanapun, ini mungkin disebabkan oleh keradangan teruk paru-paru pada tikus mincit imunisasi secara intranasal yang mungkin menjejaskan hasil tindak balas imun secara keseluruhan. Kesan adjuvan yg terdiri daripada *Lactococcus lactis* yang merembeskan tetikus IL-12 bersama dengan Lp ACERL telah terbukti dalam memberi kesan yang lebih memihak kepada keadaan calon vaksin untuk menjana lagi tindak balas imun Th1 berbanding immunisasi hanya dengan Lp ACERL sahaja. Kesan peningkatan yang memihak kepada Th1 respon dapat dilihat berdasarkan kepada hasil tindak-balas immunisasi daripada serum IgG sub-kelas dan profil cytokine daripada limpa, paru-paru dan salur gastrousus. Oleh itu, kajian ini menunjukkan bahawa vaksin yang dibangunkan iaitu Lp ACERL bersama atau tidak bersama adjuvan dapat memberi stimulasi Th1 yang diinginkan serta sangat penting sebagai penanda aras kepada kebolehan vaksin tersebut untuk menggalakkan dan mencapai tindak balas memori pelindung yang berkesan. Kesimpulanny, kajian ini membuktikan keberkesanan konsep dalam pembangunan *L. plantarum* sebagai pembawa untuk vaksin tuberkulosis bukan organisma dimodifikasi secara genetik (GMO), dan membolehkannya menjadi strategi pilihan bagi pembangunan vaksin tuberkulosis pada masa hadapan.

© COPYRIGHT UPM

ACKNOWLEDGEMENTS

I thank Allah s.w.t for His mercy and guidance that have helped me in conducting this study and in the preparation of this thesis.

I would like to express my deep appreciation and gratitude to the chairperson of my Supervisory Committee, Prof. Datin Paduka Dr. Khatijah for her relentless guidance, suggestion, encouragement and patience throughout this project and my co-supervisors Prof. Dr. Raha Abdul Rahim and Assoc. Prof. Dr. Maha Abdullah for their valuable advices. All their invaluable help is greatly appreciated.

I would like to convey my appreciation to Dr. Adelene Song Ai Lian for her assistance throughout this thesis writing process, Dr Fuzina for her help and guidance in animal handling, and Associate professor Dr Yeap Swee Keong for helping in the animal study design. I would like to convey thanks to my labmate Jeevanathan, Munir, Sarah, Azmi, Shawal, Erni, Sarah, Elina and Nini, for their help and encouragement.

I am grateful to my parents, parents-in-law, my brothers and sister for their endless support and encouragement.

Finally, I am very blessed to have my wife Farah Etillia, who has been with me from the start of my PhD journey. Her endless supports have motivated me in finishing this journey and for that I am truly grateful to her.

I would like to thank Ministry of Science, Technology and Innovation (MOSTI) for awarding me MyPhD scholarship. I offer my regards and blessings to all of those who supported me in any way in the completion of the project.

© COPYRIGHT UPM

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Khatijah Yusoff, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Raha Abdul Rahim, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

Maha Abdullah, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

____
ROBIAH BINTI YUNUS, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

© COPYRIGHT UPM

Declaration by graduate student

I hereby confirm that:
- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other
- degree at any other institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia(Research) Rules 2012;
- written permission must be obtained from supervisor and the office of DeputyVice-Chancellor (Research and Innovation) before thesis is published (in theform of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts,posters, reports, lecture notes, learning modules or any other materials asstated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia(Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti PutraMalaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: ________________________ Date: ___________________

Name and Matric No.: Anhar Danial Bin Mustafa, GS31996

© COPYRIGHT UPM

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature: ______________________________

Name of Chairman of Supervisory Committee: ______________________________

Signature: ______________________________

Name of Member of Supervisory Committee: ______________________________

Signature: ______________________________

Name of Member of Supervisory Committee: ______________________________

© COPYRIGHT UPM

TABLE OF CONTENTS

	Page
ABSTRACT	i
ABSTRAK	iii
ACKNOWLEDGEMENTS	v
APPROVAL	vi
DECLARATION	viii
LIST OF TABLES	xiv
LIST OF FIGURES	xv
LIST OF ABBREVIATIONS	xviii

CHAPTER

1 INTRODUCTION	
1.1 Introduction	1
2 LITERATURE REVIEW	
2.1 Severity and epidemiology of tuberculosis	4
2.2 Etiology of tuberculosis	5
2.2.1. Biology of Mycobacteria	5
2.2.2. Pathogenesis of pulmonary tuberculosis	6
2.2.3. The human immune response against tuberculosis	8
2.2.3.1. Assessment of specific cytokine for tuberculosis vaccine effectiveness	9
2.3 Treatment and control of tuberculosis	11
2.3.1. Anti-tuberculosis therapy	11
2.3.2. Vaccination	12
2.3.3. The BCG vaccine: Its efficacy and limitations	13
2.3.4. Current approach for tubeculosis prophylactic vaccine development	14
2.3.4.1. DNA vaccines	17
2.3.4.2. Vectored vaccines	17
2.3.4.3. Recombinant BCG	17
2.3.4.4. Subunit and fusion protein vaccines	18
2.3.4.4.1 Effect of IL-12 cytokine as adjuvant on the subunit vaccine	19
2.3.4.5. Killed, inactivated and recombinant mycobacteria	20
2.4 Experimental animal model of tuberculosis	20
2.4.1. The mouse model	21
2.4.2. The guinea pig model	21
2.4.3. The large animal model	22
2.5. Lactic acid bacteria	22
2.5.1. The physiological characteristics and taxonomy of LAB	22
2.6. Bacterial surface display system	23
2.6.1. Surface display developed for Gram-positive bacteria	24
2.6.2. Anchoring motif proteins of cell wall in LAB	28
2.6.2.1. LysM binding motif	29
2.7. Lactic acid bacteria as therapeutic molecules delivery vehicles	31

© COPYRIGHT UPM

2.7.1. *Lactococcus lactis* 31
2.7.2. *Lactobacillus plantarum* 32
2.7.3. Mucosal Immune responses to LAB displaying antigens 33
2.8. Potential and limitations of *Lactobacillus* as vaccine delivery for tuberculosis vaccine development 34

3 MATERIALS AND METHODS
3.1 Cloning of recombinant expression vector 37
3.1.1. Bacterial strains and growth conditions 37
3.1.2. *L. lactis* MG1363 genomic DNA extraction 41
3.1.3. Plasmid extraction 42
3.1.4. Agarose gel electrophoresis 42
3.1.5. Gel purification 43
3.1.6. Preparation of competent *E. coli* Rossetta cells 43
3.1.7. Construction of recombinant plasmids 43
3.1.7.1. Gene synthesis and primer design 43
3.1.7.2. PCR amplification 44
3.1.7.3. Restriction enzyme (RE) digestion and ligation of PCR products 45
3.1.7.4. Transformation into *E. coli* Rossetta competent cells 45
3.1.7.5. Screening of the recombinant plasmids 46
3.1.7.5.1. Colony PCR 46
3.1.7.5.2. Verification of the plasmid constructs 46
46
3.2 3.1.8. Growth study 47
Protein expression and extraction 47
3.2.1. N-lauryl sarcosine treatment for inclusion bodies 48
3.2.2. Protein purification using Ni^{+} affinity column 48
3.2.3. Bradford assay quantification 48
3.2.4. Protein sample preparation 49
3.2.5. SDS-PAGE analysis 49
3.2.6. Western blot analysis
3.2.7. Quantification of extracellular mIL-12 expression via 50
3.3 ELISA 50
Attachment of the fusion proteins onto *L. plantarum* cell wall 50
3.3.1. Binding stability analysis and verification 51
3.3.2. Whole cell ELISA 51
3.4 3.3.3. Immunofluorescence staining 52
3.5 Storage of vaccine candidates in -80ºC 52
Immunogenicity of the vaccine candidates 52
3.5.1. Overview of the mice study 53
3.5.2. Media 54
3.5.3. Vaccine preparation 54
3.5.4. Mouse 55
3.5.5. Intranasal and oral immunisation strategy 58
3.5.6. Determination of antibody IgG isotyping titre 59
3.5.7. Mice sacrification 59
3.5.8. Splenocytes preparation
3.5.9. Immunophenotyping of immunized mice splenocytes 60
for CD4+/CD8+ T-cell population 60

© COPYRIGHT UPM

3.5.10. Induction of splenocytes of immunized mice 60
3.5.11. Cytokine assessment 61
3.6 3.5.12. Histopathology of lungs, kidney and liver analysis 61
3.7 Statistical analysis 61
Bioinformatics analysis

4 **RESULTS**
4.1. In silico analysis for predicting potential antigenic response of ACERL, ACEL, ARL, CERL and RL 63
4.2. Construction of expression plasmids 72
4.2.1. Development of pRSF:ACERL plasmid 75
4.2.2. Development of pRSF:ACEL plasmid 79
4.2.3. Development of pRSF:ARL plasmid 81
4.2.4. Development of pRSF:CERL plasmid 85
4.2.5. Development of pRSF:RL plasmid 87
4.3. Growth study of recombinant *E. coli* constructs 90
4.4. Expression of recombinant clones 90
4.4.1. N-Lauryl Sarcosine treatment of inclusion bodies 92
4.4.2. His-tag purification of the recombinant proteins 94
4.4.3. Quantification of extracellular mIL-12 expression via ELISA 97
4.5. Verification of plasmid-expressed protein functionality 97
4.5.1 Immunoflourescene microscopy analysis on the attachement of recombinant antigens of interest interact with *L. plantarum* 99
4.5.2. Whole cell ELISA on the attachement of recombinant antigens of interest interact with *L. plantarum* 106
4.6. Effect of storage on the binding stability of the attached recombinant proteins onto *L. plantarum* cell surface 106
4.7. Immunogenicity analysis via mice animal model 109
4.7.1. Serum IgG antibody response 110
4.7.2. Immunophenotyping of immunized mice via the oral and intranasal routes 111
4.7.3. Specific IgG sub-class responses of immunized mice via the oral and intranasal routes 112
4.7.4. Specific IgA responses of immunized mice via the oral and intranasal routes 115
4.7.5. Evaluation of cytokine responses of spleen, lung and GIT after vaccination 117
4.7.6. Evaluation of cytokine response of re-stimulated splenocytes of the immunized mice 127
4.8. Histopathology 130

5 **DISCUSSION**
5.1. Construction, intracellular expression and purification of *M. tuberculosis* fusion proteins in *E. coli*
5.2. Anchoring and stability of *M. tuberculosis*-LysM fusion protein onto *L. plantarum* cell wall 133
5.3. Immunogenicity effects of Lp ACERL via oral and nasal route of administration on mice animal model 136

5.4. Immunogenicity effects of Lp ACERL co-administered with *L. lactis* secreting mIL-12 via oral and nasal route of administration on mice animal model 139

146

6 **CONCLUSION, SIGNIFICANCE OF STUDY AND FUTURE RECOMMENDATIONS**

148

REFERENCES 150
APPENDICES 166
BIODATA OF STUDENT 221
LIST OF PUBLICATIONS 222

© COPYRIGHT UPM

LIST OF TABLES

Table		Page
2.1	Tuberculosis vaccine candidates in clinical trials and their antigens	15
2.2	Characterized cell wall binding domains of several Gram-positive autolysins	24
2.3	Examples of heterologous protein anchoring in lactococcal system by utilizing native and foreign anchors	26
3.1	Bacterial strains and plasmids used/or developed in this study	38
3.2.	Primers used for amplification of the ACER, ACE, AR, CER, R0R2 and LysM genes.	44
3.3.	Immunization strategy of both oral and nasal mice Group 1 to 6	56
4.1	Secondary structural analysis of recombinant peptide proteins of ACERL, ACEL, ARL, CERL and RL by ProtParam tool analysis software	64
4.2	The predicted peptide epitopes for MHC-I immunogenicity recognition were determined using the MHC class I immunogenicity prediction software	67
4.3	The predicted peptide regions for MHC-II binding site recognition were determined using the MHC class II Binding Peptide Prediction Server	69
4.4	The predicted epitopes of designed recombinant antigen recognized immunogenically by the B cell were determined using the BCP prediction software	71
4.5	Recombinant antigen protein recoveries of ACERL, ACEL, ARL, CERL and RL from inclusion bodies (IBs) by 5% (w/v) N-lauroyl sarcosine (NLS) and its purification by Ni^{2+}-NTA affinity chromatography	95

© COPYRIGHT UPM

LIST OF FIGURES

Figure		Page
2.1	The estimated tuberculosis cases worldwide in 2014	5
2.2	Expansion of granuloma in tuberculosis	7
2.3	The host immune response in human tuberculosis infection	9
2.4	The cell surface layout of the Gram-positive bacterium containing several types of anchor proteins	29
2.5	The concept of in-trans surface display	31
3.1	Schematic diagram of a) pRSF:Duet-1 and b) pNZ8048 plasmids	40
3.2	The overall schematic process of the mice immunization and immunogenicity study	53
3.3	Microisolator cages of SPF Balb/c mice at COMeT unit of IBS, UPM	55
3.4	Administration of vaccine onto mice	57
3.5	Immunization schedule for this study	58
3.6	Harvesting of the mice organs	59
4.1	Figure of predicted tertiary structures, stability and solubility of the recombinant antigen of ACERL, ACEL, ARL, CERL and RL using RaptorX, a protein structure prediction server	65
4.2	Cloning strategy using pRSF:LysM as plasmid backbone for constructing the desired expression plasmid of interest	73
4.3	Gradient PCR of LysM gene amplification from *L. lactis* genomic DNA with flanking sequence using the LysM Fwd and Rev primers	74
4.4	Colony PCR to screen for positive transformants harbouring pRSF:LysM using specific LysM Fwd and Rev primers	74
4.5	Digestion profile of plasmid extracted from positive transformants harbouring pRSF:*LysM* (colony 3)	75
4.6	Gradient PCR for ACE gene amplification from pJET:ACE with flanking sequence using the ACE Fwd and Rev primers	76
4.7	Gradient PCR for R0R2 gene amplification from pJET:R0R2 with flanking sequence using the R0R2Fwd and Rev primers	76
4.8	Gradient PCR for ACER gene amplification from ligation mixture of fusion gene of ACER with flanking sequence using the ACER Fwd and Rev primers	77
4.9	Colony PCR to screen for positive transformants harbouring pRSF:ACERL using specific ACERLFwd and Rev primers	78
4.10	Digestion profile of plasmid extracted from positive transformants harbouring pRSF:ACERL (colony 4)	78
4.11	Gradient PCR for ACE gene amplification from pJET:ACE with flanking sequence using the ACE Fwd and Rev primers.	80
4.12	Colony PCR to screen for positive transformants harbouring pRSF:ACEL using specific ACEL Fwd and Rev primers	80
4.13	Digestion profile of plasmid extracted from positive transformants harbouring pRSF:ACEL (colony 1)	81

© COPYRIGHT UPM

4.14	Gradient PCR for Ag85B gene amplification from pJET:ACE with flanking sequence using the Ag85B Fwd and Rev primers	82
4.15	Gradient PCR amplification of R0R2 gene from pJET:R0R2 using the R0R2 Fwd and Rev primers	82
4.16	Gradient PCR for AR gene amplification from AR ligation mixture with flanking sequence using the Ag85BFwd and R0R2 Rev primers	83
4.17	Colony PCR to screen for positive transformants harbouring pRSF:ARL using specific ARL Fwd and Rev primers	84
4.18	Digestion profile of plasmid extracted from positive transformants harbouring pRSF:ARL (colony 3)	84
4.19	Gradient PCR for CERL gene amplification from pRSF:ACERL with flanking sequence using the CERL Fwd and Rev primers	85
4.20	Colony PCR to screen for positive transformants harbouring pRSF:CERL using specific CERL Fwd and Rev primers	86
4.21	Digestion profile of plasmid extracted from positive transformants harbouring pRSF: CERL (colony 2)	87
4.22	Gradient PCR for R0R2-LysM gene amplification from pRSF:ACERL with flanking sequence using specific R0R2 Fwd and LysM Rev primers	88
4.23	Colony PCR to screen for positive transformants harbouring pRSF:RL using specific RL Fwd and Rev primers	88
4.24	Digestion profile of plasmid extracted from positive transformants harbouring pRSF:RL (colony 4)	89
4.25	Growth profiles of induced *E. coli* strains after induction of 1 mM/ml of IPTG at 0.5 of OD_{600nm} grown in TB broth with kanamycin	90
4.26	Over-expression of recombinant fusion proteins of ACERL, ACEL, ARL, CERL and RL expressed from E. coli Rossetta (DE3) host via SDS-PAGE analysis	91
4.27	SDS-PAGE of soluble fraction of recombinant fusion proteins of ACERL, ACEL, ARL, CERL and RL treated with 0.2% NLS	93
4.28	Solubilization and purification of ARL inclusion bodies (IBs) using varying concentrations of N-lauroylsarcosine (NLS)	94
4.29	Soluble fractions of recombinant fusion protein of interest post-NLS of 5% treatment purified via Ni-NTA His-tag protein purification	95
4.30	Western blot analysis of crude recombinant fusion proteins of ACERL, ACEL, ARL, CERL and RL post-NLS treatment	96
4.31	Quantification of extracellular mIL-12 expression of *L. lactis* via ELISA	97
4.32	Growth profiles of *L. plantarum* Pa21 carrying antigen in MRS broth without antibiotic	98
4.33	Immunoflouroscence microscopy of *L. plantarum* interacted with ACERL protein	100
4.34	Immunoflouroscence microscopy of *L. plantarum* interacted with ACEL protein	101

© COPYRIGHT UPM

4.35	Immunoflouroscence microscopy of *L. plantarum* interacted with ARL protein	102
4.36	Immunoflouroscence microscopy of *L. plantarum* interacted with CERL protein	103
4.37	Immunofluorescence microscopy of *L. plantarum* interacted with RL protein	104
4.38	Immunofluorescence microscopy of *L. plantarum* interacted with 1x PBS as negative control	105
4.39	Stability analysis of the binding of the fusion proteins onto the cell surface *L. plantarum* via whole cell ELISA for a four-day period	106
4.40	Stability analysis of attached fusion proteins of interest onto cell surface of *L. plantarum* stored at -80°C for 4-week period with 20% glycerol	107
4.41	Stability analysis of attached fusion proteins of interest onto cell surface of *L. plantarum* stored at -80°C for 4-week period without glycerol	108
4.42	Time-course of the serum total IgG antibody response against Lp ACERL, Lp ACERL/LcIL-12, ACERL, LcIL-12, Lp and PBS immunization	110
4.43	Immunophenotyping of mice splenocytes	112
4.44	Specific antibody isotype secretions in prophylactic mice, immunized as outline in Table 3.1	113
4.45	Mucosal specific IgA of spleen, lung, GIT, feacal and serum	115
4.46	Cytokine response of spleen from immunized Balb/c mice of oral and nasal groups	118
4.47	Cytokine response of lung from immunized Balb/c mice of oral and nasal groups	121
4.48	Cytokine response of GIT from immunized Balb/c mice of oral and nasal groups	125
4.49	Cytokine response of re-stimulated splenocytes with ACERL antigen after 48 hours from immunized Balb/c mice of the oral and nasal groups	128
4.50	Lungs of immunized mice from oral and nasal groups	130
4.51	H&E stained lungs of immunized mice from oral and nasal groups	132

© COPYRIGHT UPM

LIST OF ABBREVIATIONS

%	percentage
°C	degree Celsius
μg	microgram
μl	microliter
Abs	absorbances
ACMA	N-acetylglucosaminidase
APC	antigen presenting cell
BCG	Bacilli Calmette–Guérin
bp	base pair
BSA	bovine serum albumin
CD4	cluster of differentiation 4
CD8	cluster of differentiation 8
CFU	colony forming unit
CO_2	cytotoxic T-lymphocyte-associated protein 4
DAB	3,3'-diaminobenzidine
DCs	dendritic cells
dH_20	distilled water
DNA	deoxyribonucleic acid
ELISA	enzyme-linked immunosorbent assay
FITC	Fluorescein isothiocyanate
GALT	gut-associated lymphoid tissue
GFP	green fluorescent protein
GM	genetically modified
GMO	genetically modified organism
GRAS	generally regarded as safe
h	hour
His (H)	histidine
HIV	human immunodeficiency virus
HRP	horseradish peroxidase
IBs	inclusion bodies
IFN-γ	interferon gamma,
IL-10	interleukin 10
IL-12	interleukin 12
IL-2	interleukin 2
IL-4	interleukin 4
IL-6	interleukin 6
kDa	kilo Dalton
L	litre
LAB	lactic acid bacteria
LAM	Lipoarabinomannan,
LB	Luria Bertani
LTA	lipoteichoic acid
LysM	Lysin motif
M	molarity
mAb	monoclonal antibody
MALT	mucosa-associated lymphoid tissue
MCS	multiple cloning site

© COPYRIGHT UPM

MDR-TB	multi-drug resistance tuberculosis
Met (M)	methionine
MHC-I	major histocompatibility complex I
MHC-II	major histocompatibility complex II
min	minute
mM	millimolar
MRS	de Man, Rogosa and Sharp agar
MTB	*Mycobacterium tuberculosis*
MVA85A	modified vaccinia Ankara 85A
NAG	N-Acetylglucosamine
NaOH	sodium hyroxide
ng	nanogram
NK	Natural killer cell
NLS	N-lauryl sarcosine
nm	nanometer
NO	nitrogen oxide
PBMC	peripheral blood mononuclear cell
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PG	peptidoglycan
pH	potential of hydrogen
PIM	phosphatidylinositol mannoside
PVDF	Polyvinylidene fluoride
RE	restriction enzyme
RNA	ribonucleic acid
ROS	reactive oxygen species.
RPMI	Roswell Park Memorial Institute medium
RT	room temperature
SDS	sodium dodecyl sulfate
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
TAE	tris-acetate EDTA
Taq	*Thermus aquaticus*
TB	terrific Broth
Tc	T cytotoxic cell
TCA	trichloroacetic acid
TEMED	tetramethyl-ethylene diamine
Th	T helper
Th1	type 1 T helper
Th17	type 17 T helper
Th2	type 2 T helper
Tm	melting temperature
TNF-α	tumor necrosis factor alpha
Treg	T regulatory
TTFC	tetanus toxin fragment C
UV	ultra-violet
v	volt
v/v	volume per volume
w/v	weight per volume

© COPYRIGHT UPM

CHAPTER 1

INTRODUCTION

1.1 Introduction

Tuberculosis is one of the most common and deadliest infectious disease apart from ebola, malaria and the human immunodeficiency virus (HIV) syndrome. Almost a third of the world's population has been latently infected with *Mycobacterium tuberculosis*, the causative agent for tuberculosis. It was estimated that in 2014, out of the 9.6 million infected cases, 1.5 million tuberculosis related-deaths were reported worldwide (WHO, 2015); over 95% of which occur in low- and middle-income countries, predominantly in Africa, South East Asia and Eastern Europe (Pareek *et al.*, 2016). Although drugs are available for the treatmentof these patients, these tend to be relatively expensive and the long duration of 6 months required for the treatment has led to poor compliance by the patients. This indivertibly gives rise to the emergence of multiple drug resistant strains. The disease is being prevented and controlled by the Bacille Calmette Guérin (BCG) vaccine, the only viable vaccine approved for human usage since 1921. Nevertheless, the protective efficacy of the BCG vaccine has been highly variable and sub-standard (0-80%) especially in the endemic tropical and sub-tropical regions, due to pre-existing immune responses to the vaccine (WHO, 2010). Therefore, there is a strong need for a new and effective tuberculosis vaccine to be developed either as a replacement or as a booster in order to overcome the limitations from the BCG vaccine.

At present, research for novel tuberculosis vaccination strategies are intensely focused on the attenuated mycobacterial vaccine as well as subunit vaccine, and viral vector vaccines (Delogu and Fadda, 2009). Among these approaches, the subunit vaccine approach has shown to give the most promising outcome as indicated by its success in clinical trials stage I or II (Sarhan, 2010). Nonetheless, the progress of these vaccines are often hampered by concerns on the safety aspects with regards to genetically modified organism (GMO)-type vaccine, induction of negative side-effects, and failure to ensure significant protective immune responses (Clark and Cassidy-Hanley, 2005). For example, although the attenuated mycobacterial and viral vector vacciness often showed strong protective immune response, the safety concern on the possible reversion to a pathogenic and virulence state has become one of the main issues for these vaccine strategies (Minor, 2015). In contrast, a subunit vaccine is regarded to have a higher safety approval but typically suffers from the lack of any significant protective immune response and requires adjuvant in order to improve dramatically the immunogenicity response (Mohan *et. al*., 2013).

A different approach towards developing an effective tuberculosis vaccine is to use the probiotic lactic acid bacteria (LAB) as a mucosal delivery vehicle to exhibit the antigenic protein(s) on their cell surface. This strategy has already been successfully performed with promising results in giving protective immunity against rotavirus, group B streptococcus, HPV16-induced tumors, tetanus, chicken anemia virus and enterovirus

© COPYRIGHT UPM

(Wells and Mercenier, 2008). The growing interest in the use of the LAB protein surface display system for mucosal vaccination purposes is due to strong requirement for effective strategies in delivering vaccine antigens, microbiocides and therapeutics to the mucosal tissues (Wells and Mercenier, 2008). The rationale of developing a tuberculosis vaccine through the mucosal route is due to the *M. tuberculosis* target entry site at the mucosal lining of the respiratory tract. By focusing on this route for vaccine administration, effective protection gain by the mucosal cells against the pathogen can be achieved primarily by the enhancement of mucosal cells to vaccine interaction whilst having reduced potential side effects when compared to systemic routes of administration (Czerkinsky *et al*., 1999). This is because studies have shown that protection against mycobacterial infection were conferred by mucosal immunity that induces both mycobacterial-specific T helper cells-1 (Th-1) and secretory IgA responses which are the key immune response against *M. tuberculosis* (Badell *et al*., 2009; Hockey and Misra, 2011). Moreover, this approach provides painless and easy vaccine administration with higher compliance rate than the other known invasive administration methods.

In this project, candidate mucosal tuberculosis vaccines utilizing *in-trans* protein surface display system of *Lactobacillus plantarum* carrying and displaying five combinations of *M. tuberculosis* subunit epitope antigens (Ag85B, CFP-10, ESAT-6, Rv0475 and Rv2031c) fused to the LysM anchor motif, designated as ACERL, ACEL, ARL, CERL and RL were constructed, cloned and expressed in *Esherichia coli* Rossetta expression host. Subsequently, these expressed fusion proteins were harvested and purified before being externally attached onto the cell wall of *L. plantarum*. This strategy of using an independent expression host for protein expression and external attachment of the expressed protein onto the cell wall of the intended bacterial carrier is referred as the *in-trans* surface display system concept. The recombinant *M. tuberculosis* antigen-LysM protein fusions were studied for their functionality in their binding capability to the cell wall of *L. plantarum* and their immunogenicity effects based on mouse animal model. The effect of adding adjuvant consisting of *Lactococcus lactis* secreting IL-12 co-administered with the candidate vaccines was also determined as it has the potential to induce a more favorable condition for the candidate vaccine to further generate significant and improved protective immune responses.

The delivery system in this project is novel compared to other tuberculosis vaccination approaches since it uses non-pathogenic and non-engineered *L. plantarum* as the carrier vehicle to surface display *M. tuberculosis* antigens. Moreover, the *L. plantarum* has a GRAS status (Raha *et al*., 2005) that provides an attractive option compared to the use of other mucosal delivery systems, such as liposomes, microparticles and attenuated pathogens (Brandtzaeg, 2003). *L. plantarum* also poses adjuvant properties that have showed to help skew the mix Th1/Th2 immune response commonly elicited by *M. tuberculosis* antigens to a more preferred Th1-type cellular immune response, thus providing strong protective memory immunity against tuberculosis (Ghadimi *et al*., 2010). However, even with the advantages of this unique strategy, there are no reports yet that have fully investigated its potential for tuberculosis vaccine development specifically in the use of *in-trans* surface display system of *L. plantatum*. Thus, this project is intended to determine the potential response using LAB of *L. plantarum* as non-GMO vaccine carrier via the *in-trans* surface display system for the development of candidate mucosal tuberculosis vaccine.

© COPYRIGHT UPM

The hypotheses of this project are as follow:-

1. The constructed candidate vaccine consisting of LysM anchor protein fused with a combination of selected *M. tuberculosis* antigen epitopes of Ag85B, CFP-10, ESAT-6, Rv2031c and Rv0475, attached onto the cell wall of *L. plantarum* when administered via the oral and nasal route of mouse animal model will stimulate effective immune response that favour the Th1 type response which is a key indicator for potential protective memory response;

2. The use of *L. lactis* secreating mouse IL-12 as an adjuvant co-administered with the constructed candidate vaccine via the oral and nasal route in mouse animal model will further improve the immune response to favour the Th1 type response as compared to the immune response outcome from the administration of candidate vaccine only.

The project specific objectives are:-

1. To construct candidate vaccines consisting of LysM anchor protein fused with a combination of selected *M. tuberculosis* antigen epitopes of Ag85B, CFP-10, ESAT-6, Rv2031c and Rv0475 in *E. coli* Rosetta expression-based system;
2. To study the binding capability of the *M. tuberculosis* antigens-LysM protein fusion onto the cell wall surface of *L. plantarum* Pa21;
3. To compare the immunogenicity effect of the candidate vaccines administered via oral and intranasal route using a mouse animal model; and
4. To determine the immunogenicity effect of the candidate vaccine co-administered with an adjuvant consisting of *L. lactis* secreting mouse IL-12 on mouse animal model.

© COPYRIGHT UPM

REFERENCES

Adetifa, I. M., Ota, M. O. C., Jeffries, D. J., Lugos, M. D., Hammond, A. S., Battersby, N. J., Hill, P. C. (2013). Interferon-gamma ELISPOT as a biomarker of treatment efficacy in latent tuberculosis infection: a clinical trial. *American Journal of Respiratory and Critical Care Medicine*, *187*(4), 439–445.

Agger, E. M., & Andersen, P. (2002). A novel TB vaccine; towards a strategy based on our understanding of BCG failure. *Vaccine*, *21*(1–2), 7–14.

Aggerbeck, H., & Madsen, S. M. (2006). Safety of ESAT-6. *Tuberculosis (Edinburgh, Scotland)*, *86*(5), 363–73.

Andersen, P., & Doherty, T. M. (2005a). TB subunit vaccines - Putting the pieces together. *Microbes and Infection*, *7*(5–6), 911–921. for

Andersen, P., & Doherty, T. M. (2005b). The success and failure of BCG - implications for a novel tuberculosis vaccine. *Nat Rev Micro*, *3*(8), 656–662.

Annan, N. T., Borza, a. D., & Hansen, L. T. (2008). Encapsulation in alginate-coated gelatin microspheres improves survival of the probiotic *Bifidobacterium adolescentis* 15703T during exposure to simulated gastro-intestinal conditions. *Food Research International*, *41*(2), 184–193.

Arai, R., Ueda, H., Kitayama, A., Kamiya, N., & Nagamune, T. (2001). Design of the linkers which effectively separate domains of a bifunctional fusion protein. *Protein Engineering*, *14*(8), 529–32.

Badell, E., Nicolle, F., Clark, S., Majlessi, L., Boudou, F., Martino, A., Winter, N. (2009). Protection against tuberculosis induced by oral prime with *Mycobacterium bovis* BCG and intranasal subunit boost based on the vaccine candidate Ag85B-ESAT-6 does not correlate with circulating IFN-γ producing T-cells. *Vaccine*, *27*(1), 28–37.

Bahey-El-Din, M. (2012). *Lactococcus lactis*-based vaccines from laboratory bench to human use: An overview. *Vaccine*, *30*(4), 685–690.

Bahey-El-Din, M., Gahan, C. G. M., & Griffin, B. T. (2010). *Lactococcus lactis* as a cell factory for delivery of therapeutic proteins. *Current Gene Therapy*, *10*(1), 34–45.

Baker, D. G. (1998). Natural pathogens of laboratory mice, rats, and rabbits and their effects on research. *Clinical Microbiology Reviews*, *11*(2), 231–266.

Baradaran, A., Yusoff, K., Shafee, N., & Rahim, R. A. (2016). Newcastle disease virus hemagglutinin neuraminidase as a potential cancer targeting agent. *Journal of Cancer*, *7*(4), 462–466.

Barker, L. F., Brennan, M. J., Rosenstein, P. K., & Sadoff, J. C. (2009). Tuberculosis vaccine research: the impact of immunology. *Current Opinion in Immunology*, *21*(3), 331–338.

Barone, F., Patel, P., Sanderson, J. D., & Spencer, J. (2009). Gut-associated lymphoid tissue contains the molecular machinery to support T-cell-dependent and T-cell-independent class switch recombination. *Mucosal Immunol*, *2*(6), 495–503.

Batista-Duharte, A., Portuondo, D., Pérez, O., & Carlos, I. Z. (2014). Systemic immunotoxicity reactions induced by adjuvanted vaccines. *International Immunopharmacology*, *20*(1), 170–180.

Bawa, A. S., & Anilakumar, K. R. (2013). Genetically modified foods: safety, risks and public concerns—a review. *Journal of Food Science and Technology*, *50*(6), 1035–1046.

Bermúdez-Humarán, L. G., Innocentin, S., Lefèvre, F., Chatel, J.-M., & Langella, P. (2009). Development of mucosal vaccines based on lactic acid bacteria. *Prebiotics and Probiotics Science and Technology*, 1099–1122.

© COPYRIGHT UPM

Bermúdez-Humarán, L. G., Kharrat, P., Chatel, J.-M., & Langella, P. (2011). Lactococci and lactobacilli as mucosal delivery vectors for therapeutic proteins and DNA vaccines. *Microbial Cell Factories*, *10 Suppl 1*(Suppl 1), S4.

Beveridge, N. E. R., Fletcher, H. A., Hughes, J., Pathan, A. A., Scriba, T. J., Minassian, A., McShane, H. (2008). A comparison of IFN-γ detection methods used in tuberculosis vaccine trials. *Tuberculosis*, *88*(6), 631–640.

Blazevic, A., Eickhoff, C. S., Stanley, J., Buller, M. R., Schriewer, J., Kettelson, E. M., & Hoft, D. F. (2014a). Investigations of TB vaccine-induced mucosal protection in mice. *Microbes and Infection / Institut Pasteur*, *16*(1), 73–9.

Blazevic, A., Eickhoff, C. S., Stanley, J., Buller, M. R., Schriewer, J., Kettelson, E. M., & Hoft, D. F. (2014b). Investigations of TB vaccine-induced mucosal protection in mice. *Microbes and Infection / Institut Pasteur*, *16*(1), 73–9.

Boshoff, H. I. M., & Barry, C. E. 3rd. (2005). Tuberculosis - metabolism and respiration in the absence of growth. *Nature Reviews. Microbiology*, *3*(1), 70–80.

Bothamley, G. (2008). Management of tuberculosis. *British Journal of Hospital Medicine (London, England : 2005)*, *69*(12), 699–704.

Brandtzaeg, P. (2009). Mucosal immunity: Induction, dissemination, and effector functions. *Scandinavian Journal of Immunology*, *70*(6), 505–515.

Bronte, V., & Pittet, M. J. (2013). The spleen in local and systemic regulation of immunity. *Immunity*, *39*(5), 806–818.

Buist, G., Steen, A., Kok, J., & Kuipers, O. P. (2008). LysM, a widely distributed protein motif for binding to (peptido)glycans. *Molecular Microbiology*, *68*(4), 838–847.

Burchill, M. a, Tamburini, B. a, Pennock, N. D., White, J. T., Kurche, J. S., & Kedl, R. M. (2013). T cell vaccinology: exploring the known unknowns. *Vaccine*, *31*(2), 297–305.

Burgess, R. R. (2009). *Refolding solubilized inclusion body proteins. Methods in enzymology* (1st ed., Vol. 463). Elsevier Inc.

Cambier, C. J., Falkow, S., & Ramakrishnan, L. (2014). Host evasion and exploitation schemes of *Mycobacterium tuberculosis*. *Cell*.

Camus, J.-C., Pryor, M. J., Medigue, C., & Cole, S. T. (2002). Re-annotation of the genome sequence of *Mycobacterium tuberculosis* H37Rv. *Microbiology (Reading, England)*, *148*(Pt 10), 2967–2973.

Cao, J., Zhang, X., Gong, Y., Zhang, Y., Cui, Y., Lai, X., & Yin, Y. (2013). Protection against pneumococcal infection elicited by immunization with multiple pneumococcal heat shock proteins. *Vaccine*, *31*(35), 3564–3571.

Cavalcanti, Y. V. N., Brelaz, M. C. A., Neves, J. K. de A. L., Ferraz, J. C., & Pereira, V. R. A. (2012). Role of TNF-Alpha, IFN-Gamma, and IL-10 in the Development of Pulmonary Tuberculosis. *Pulmonary Medicine*, *2012*, 745483.

Checkley, A. M., & McShane, H. (2011). Tuberculosis vaccines: progress and challenges. *Trends in Pharmacological Sciences*, *32*(10), 601–6.

Chen, K., & Cerutti, A. (2010). Vaccination strategies to promote mucosal antibody responses. *Immunity*, *33*(4), 479–91.

Cheng, Y., Jope, R. S., & Beurel, E. (2015). A pre-conditioning stress accelerates increases in mouse plasma inflammatory cytokines induced by stress. *BMC Neuroscience*, *16*(1), 31.

Cheun, H. I., Kawamoto, K., Hiramatsu, M., Tamaoki, H., Shirahata, T., Igimi, S., & Makino, S. I. (2004). Protective immunity of SpaA-antigen producing *Lactococcus lactis* against Erysipelothrix rhusiopathiae infection. *J Appl Microbiol*, *96*.

Childers, N. K., Miller, K. L., Tong, G., Llarena, J. C., Greenway, T., Ulrich, J. T., & Michalek, S. M. (2000). Adjuvant activity of Monophosphoryl Lipid A for nasal and oral immunization with soluble or liposome-associated antigen. *Infection and*

© COPYRIGHT UPM

Immunity, *68*(10), 5509–5516.
Christy, A. J., Dharman, K., Dhandapaani, G., Palaniyandi, K., Gupta, U. D., Gupta, P., Narayanan, S. (2012). Epitope based recombinant BCG vaccine elicits-specific Th1 polarized immune responses in BALB/c mice. *Vaccine*, *30*(7), 1364–70.
Clark, T. G., & Cassidy-Hanley, D. (2005). Recombinant subunit vaccines: potentials and constraints. *Developments in Biological Standardization*, *121*, 153–63.
Cole, S. T., Brosch, R., Parkhill, J., Garnier, T., Churcher, C., Harris, D., Barrell, B. G. (1998). Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. *Nature*, *393*(6685), 537–544.
Cooper, A. M. (2009). T cells in mycobacterial infection and disease. *Current Opinion in Immunology*, *21*(4), 378–384.
Cooper, A. M., Adams, L. B., Dalton, D. K., Appelberg, R., & Ehlers, S. (2002). IFN-gamma and NO in mycobacterial disease: new jobs for old hands. *Trends in Microbiology*, *10*(5), 221–226.
Cooper, A. M., Solache, A., & Khader, S. A. (2007). Interleukin-12 and tuberculosis: an old story revisited. *Current Opinion in Immunology*, *19*(4), 441–447.
Cortes-perez, N. G., Adel-patient, K., Langella, P., & Berm, L. G. (2007). Influence of the route of immunization and the nature of the bacterial vector on immunogenicity of mucosal vaccines based on lactic acid bacteria, *25*, 6581–6588.
Cortes-Perez, N. G., Ah-Leung, S., Bermúdez-Humarán, L. G., Corthier, G., Wal, J.-M., Langella, P., & Adel-Patient, K. (2007). Intranasal coadministration of live lactococci producing interleukin-12 and a major cow's milk allergen inhibits allergic reaction in mice. *Clinical and Vaccine Immunology : CVI*, *14*(3), 226–33.
Corthésy, B., Boris, S., Isler, P., Grangette, C., & Mercenier, A. (2005). Oral immunization of mice with Lactic Acid Bacteria producing *Helicobacter pylori* Urease B subunit partially protects against challenge with *Helicobacter felis*. *The Journal of Infectious Diseases*, *192*(8), 1441–1449.
da Costa, C., Walker, B., & Bonavia, A. (2015). Tuberculosis Vaccines – state of the art, and novel approaches to vaccine development. *International Journal of Infectious Diseases*, *32*, 5–12.
da Silva, A. J., Zangirolami, T. C., Novo-Mansur, M. T. M., Giordano, R. de C., & Martins, E. A. L. (2014). Live bacterial vaccine vectors: An overview. *Brazilian Journal of Microbiology*, *45*(4), 1117–1129.
Daniel, C., Roussel, Y., Kleerebezem, M., & Pot, B. (2011). Recombinant lactic acid bacteria as mucosal biotherapeutic agents. *Trends in Biotechnology*, *29*(10), 499–508.
Daniel, T. M. (2006). The history of tuberculosis. *Respiratory Medicine*, *100*(11), 1862–1870.
Davitt, C. J. H., & Lavelle, E. C. (2015). Delivery strategies to enhance oral vaccination against enteric infections. *Advanced Drug Delivery Reviews*, *91*, 52–69.
Deng, Y., Bao, L., & Yang, X. (2011). Evaluation of immunogenicity and protective efficacy against *Mycobacterium tuberculosis* infection elicited by recombinant *Mycobacterium bovis* BCG expressing human Interleukin-12p70 and Early Secretory Antigen Target-6 fusion protein. *Microbiology and Immunology*, *55*(11), 798–808.
Derrick, S. C., Yabe, I. M., Yang, A., Kolibab, K., Hollingsworth, B., Kurtz, S. L., & Morris, S. (2013). Immunogenicity and protective efficacy of novel *Mycobacterium tuberculosis* antigens. *Vaccine*, *31*(41), 4641–6.
Detmer, A., & Glenting, J. (2006). Live bacterial vaccines--a review and identification of potential hazards. *Microbial Cell Factories*, *5*(iii), 23.
Dey, B., Jain, R., Khera, A., Rao, V., Dhar, N., Gupta, U. D., Tyagi, A. K. (2009).

© COPYRIGHT UPM

Boosting with a DNA vaccine expressing ESAT-6 (DNAE6) obliterates the protection imparted by recombinant BCG (rBCGE6) against aerosol *Mycobacterium tuberculosis* infection in guinea pigs. *Vaccine*, *28*(1), 63–70.

Dharmadhikari, A. S., & Nardell, E. A. (2008). What animal models teach humans about tuberculosis. *American Journal of Respiratory Cell and Molecular Biology*, *39*(5), 503–508.

Dlugovitzky, D., Notario, R., Martinel-Lamas, D., Fiorenza, G., Farroni, M., Bogue, C., Stanford, J. (2010). Immunotherapy with oral, heat-killed, *Mycobacterium vaccae* in patients with moderate to advanced pulmonary tuberculosis. *Immunotherapy*, *2*(2), 159–169.

Dogra, S., & Khullar, G. (2013). Tumor necrosis factor-α antagonists: Side effects and their management. *Indian Journal of Dermatology, Venereology, and Leprology*, *79*(7), 35–46.

Doherty, T. M., Olsen, A. W., Pinxteren, L. Van, & Andersen, P. (2002). Oral Vaccination with subunit vaccines protects animals against aerosol infection with *Mycobacterium tuberculosis*, *70*(6), 3111–3121.

Doherty, T. M., & Rook, G. (2016). Progress and hindrances in tuberculosis vaccine development. *The Lancet*, *367*(9514), 947–949.

Doitsh, G., Galloway, N. L. K., Geng, X., Yang, Z., Monroe, K. M., Zepeda, O., Greene, W. C. (2014). Pyroptosis drives CD4 T-cell depletion in HIV-1 infection. *Nature*, *505*(7484), 509–514.

Dou, J., Tang, Q., Yu, F., Yang, H., Zhao, F., Xu, W., Wang, Y. (2010). Investigation of immunogenic effect of the BCG priming and Ag85A- GM-CSF boosting in Balb/c mice model. *Immunobiology*, *215*(2), 133–42. *192*(8), 1441–1449.

Du, Z., & Uversky, V. N. (2017). A comprehensive survey of the roles of highly disordered proteins in type 2 diabetes. *International Journal of Molecular Sciences*. *18*(10), 1-44.

Ehlers, S., & Schaible, U. E. (2012). The granuloma in tuberculosis: Dynamics of a host-pathogen collusion. *Frontiers in Immunology*, *3*(1).

Esvaran, M., & Conway, P. L. (2016). Factors that influence the immunological adjuvant effect of *Lactobacillus fermentum* PC1 on specific immune responses in mice to orally administered antigens. *Vaccines*, *4*(3), 24.

Evans, T. G., Brennan, M. J., Barker, L., & Thole, J. (2013). Preventive vaccines for tuberculosis. *Vaccine*, *31 Suppl 2*, B223-6.

Ferrante, A., Beard, L. J., & Roberton, D. M. (1991). IgG subclass deficiency. *Pediatric Allergy and Immunology*, *2*(2), 49–62.

Flynn, J. L. (2006). Lessons from experimental *Mycobacterium tuberculosis* infections. *Microbes and Infection / Institut Pasteur*, *8*(4), 1179–1188.

Fonseca, F., Marin, M., & Morris, G. J. (2006). Stabilization of Frozen *Lactobacillus delbrueckii* subsp. bulgaricus in glycerol suspensions: Freezing kinetics and storage temperature effects. *Applied and Environmental Microbiology* , *72*(10), 6474–6482.

Forrellad, M. a, Klepp, L. I., Gioffré, A., Sabio y García, J., Morbidoni, H. R., de la Paz Santangelo, M., Bigi, F. (2013). Virulence factors of the *Mycobacterium tuberculosis* complex. *Virulence*, *4*(1), 3–66.

Francis, V. G., Majeed, M. A., & Gummadi, S. N. (2012). Recovery of functionally active recombinant human phospholipid scramblase 1 from inclusion bodies using N-lauroyl sarcosine. *Journal of Industrial Microbiology and Biotechnology*, *39*(7), 1041–1048.

Frederick C. Neidhardt, John L. Ingraham, M. S. (1990). *Physiology of the Bacterial Cell: A Molecular Approach*. *Biochemical Education* (Vol. 20). Headington Hill

© COPYRIGHT UPM

Hall.
Fredriksen, L., Kleiveland, C. R., Olsen Hult, L. T., Lea, T., Nygaard, C. S., Eijsink, V. G. H., & Mathiesen, G. (2012). Surface Display of N-Terminally Anchored Invasin by *Lactobacillus plantarum* Activates NF-κB in Monocytes. *Applied and Environmental Microbiology* , *78*(16), 5864–5871.
Gevers, D., Huys, G., & Swings, J. (2001). Applicability of rep-PCR fingerprinting for identification of Lactobacillus species. *FEMS Microbiology Letters*, *205*(1), 31–36.
Ghadimi, D., de Vrese, M., Heller, K. J., & Schrezenmeir, J. (2010). Lactic acid bacteria enhance autophagic ability of mononuclear phagocytes by increasing Th1 autophagy-promoting cytokine (IFN-gamma) and nitric oxide (NO) levels and reducing Th2 autophagy-restraining cytokines (IL-4 and IL-13) in response to Mycobacterium. *International Immunopharmacology*, *10*(6), 694–706.
Ghaffar, T., Irshad, M., Anwar, Z., Aqil, T., Zulifqar, Z., Tariq, A., Mehmood, S. (2014). Recent trends in lactic acid biotechnology: A brief review on production to purification. *Journal of Radiation Research and Applied Sciences*, *7*(2), 222–229.
Gill, H. S., & Rutherfurd, K. J. (2001). Viability and dose-response studies on the effects of the immunoenhancing lactic acid bacterium *Lactobacillus rhamnosus* in mice. *British Journal of Nutrition* (Vol. 86).
Gillis, T. P., Tullius, M. V, & Horwitz, M. A. (2014). rBCG30-induced immunity and cross-protection against *Mycobacterium leprae* challenge are enhanced by boosting with the *Mycobacterium tuberculosis* 30-kilodalton antigen 85B. *Infection and Immunity*, *82*(9), 3900–3909.
Grangette, C., Mu, H., Hols, P., Goudercourt, D., Delcour, J., Turneer, M., & Mercenier, A. (2004). Enhanced mucosal delivery of antigen with cell wall mutants of lactic acid bacteria, *Infection Immunity, 72*(5), 2731–2737.
Grangette, C., Müller-alouf, H., Geoffroy, M., Goudercourt, D., Turneer, M., & Mercenier, A. (2002). Protection against tetanus toxin after intragastric administration of two recombinant lactic acid bacteria : impact of strain viability and in vivo persistence.*Vaccine, 20*, 3304–3309.
Grangette, C., Müller-Alouf, H., Goudercourt, D., Geoffroy, M.-C., Turneer, M., & Mercenier, A. (2001). Mucosal immune responses and protection against tetanus toxin after intranasal immunization with recombinant *Lactobacillus plantarum*. *Infection and Immunity* , *69*(3), 1547–1553.
Griffith, D. E., & Kerr, C. M. (1996). Tuberculosis: disease of the past, disease of the present. *Journal of Perianesthesia Nursing : Official Journal of the American Society of PeriAnesthesia Nurses / American Society of PeriAnesthesia Nurses*, *11*(4), 240–5.
Grover, A., Ahmed, M. F., Singh, B., Verma, I., Sharma, P., & Khuller, G. K. (2006). A multivalent combination of experimental antituberculosis DNA vaccines based on Ag85B and regions of difference antigens. *Microbes and Infection / Institut Pasteur*, *8*(9–10), 2390–9.
Gupta, U. D., & Katoch, V. M. (2005). Animal models of tuberculosis. *Tuberculosis (Edinburgh, Scotland)*, *85*(5–6), 277–293.
Hall, L. J., Clare, S., Pickard, D., Clark, S. O., Kelly, D. L. F., El Ghany, M. A., Dougan, G. (2009). Characterisation of a live Salmonella vaccine stably expressing the *Mycobacterium tuberculosis* Ag85B-ESAT6 fusion protein. *Vaccine*, *27*(49), 6894–904.
Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, *41*, 95–98.

© COPYRIGHT UPM

Harris, J., De Haro, S. A., Master, S. S., Keane, J., Roberts, E. A., Delgado, M., & Deretic, V. (2007). T Helper 2 Cytokines inhibit autophagic control of intracellular *Mycobacterium tuberculosis*. *Immunity*, *27*(3), 505–517.

Harris, J., & Keane, J. (2010). How tumour necrosis factor blockers interfere with tuberculosis immunity. *Clinical and Experimental Immunology*, *161*(1), 1–9.

Harris, J., Master, S. S., De Haro, S. A., Delgado, M., Roberts, E. A., Hope, J. C., Deretic, V. (2009). Th1–Th2 polarisation and autophagy in the control of intracellular mycobacteria by macrophages. *Veterinary Immunology and Immunopathology*, *128*(1–3), 37–43.

Hatherill, M., Adams, V., Hughes, J., De Kock, M., Mavakla, W., Pienaar, B., Hanekom, W. A. (2009, July). The potential impact of helminth infection on trials of novel tuberculosis vaccines. *Vaccine*. Netherlands, Netherlands.

Hazebrouck, S., Oozeer, R., Adel-Patient, K., Langella, P., Rabot, S., Wal, J. M., & Corthier, G. (2006). Constitutive delivery of bovine β-lactoglobulin to the digestive tracts of gnotobiotic mice by engineered *Lactobacillus casei*. *Applied and Environmental Microbiology*, *72*(12), 7460–7467.

Heilig, H. G. H. J., Zoetendal, E. G., Vaughan, E. E., Marteau, P., Akkermans, A. D. L., & de Vos, W. M. (2002). Molecular diversity of Lactobacillus spp. and other lactic acid bacteria in the human intestine as determined by specific amplification of 16S ribosomal DNA. *Applied and Environmental Microbiology*, *68*(1), 114–123.

Hett, E. C., & Rubin, E. J. (2008). Bacterial growth and cell division: a mycobacterial perspective. *Microbiology and Molecular Biology Reviews* , *72*(1), 126–156.

Hill, P. C., Jackson-sillah, D., Fox, A., Franken, K. L. M. C., Lugos, M. D., Jeffries, D. J., Brookes, R. H. (2005). ESAT-6 / CFP-10 fusion protein and peptides for optimal diagnosis of *Mycobacterium tuberculosis* infection by Ex Vivo Enzyme-Linked Immunospot Assay in the Gambia, *43*(5), 2070–2074.

Hjelholt, A., Christiansen, G., Sørensen, U. S., & Birkelund, S. (2013). IgG subclass profiles in normal human sera of antibodies specific to five kinds of microbial antigens. *Pathogens and Disease*, *67*(3), 206–213.

Hoft, D. F., Blazevic, A., Abate, G., Hanekom, W. A., Kaplan, G., Soler, J. H., Horwitz, M. A. (2008). A new recombinant bacille Calmette-Guerin vaccine safely induces significantly enhanced tuberculosis-specific immunity in human volunteers. *The Journal of Infectious Diseases*, *198*(10), 1491–1501.

Hokey, D. a, & Misra, A. (2011). Aerosol vaccines for tuberculosis: a fine line between protection and pathology. *Tuberculosis (Edinburgh, Scotland)*, *91*(1), 82–5.

Horwitz, M. A., Harth, G., Dillon, B. J., & Maslesa-Galic', S. (2000). Recombinant bacillus calmette-guerin (BCG) vaccines expressing the *Mycobacterium tuberculosis* 30-kDa major secretory protein induce greater protective immunity against tuberculosis than conventional BCG vaccines in a highly susceptible animal model. *Proceedings of the National Academy of Sciences of the United States of America*, *97*(25), 13853–13858.

Horwitz, M. A., Lee, B. W., Dillon, B. J., & Harth, G. (1995). Protective immunity against tuberculosis induced by vaccination with major extracellular proteins of *Mycobacterium tuberculosis*. *Proceedings of the National Academy of Sciences of the United States of America*, *92*(5), 1530–1534.

Hugentobler, F., Di Roberto, R. B., Gillard, J., & Cousineau, B. (2012). Oral immunization using live *Lactococcus lactis* co-expressing LACK and IL-12 protects BALB/c mice against Leishmania major infection. *Vaccine*, *30*(39), 5726–32.

Hunter, R. L. (2017). Pathology of post primary tuberculosis of the lung: An illustrated critical review. *Tuberculosis*, *91*(6), 497–509.

© COPYRIGHT UPM

Hur, Y.-G., Kang, Y. A., Jang, S.-H., Hong, J. Y., Kim, A., Lee, S. A., Cho, S.-N. (2015). Adjunctive biomarkers for improving diagnosis of tuberculosis and monitoring therapeutic effects. *Journal of Infection*, *70*(4), 346–355.

Jefferis, R., & Kumararatne, D. S. (1990). Selective IgG subclass deficiency: quantification and clinical relevance. *Clinical and Experimental Immunology*, *81*(3), 357–67.

Junqueira-Kipnis, A. P., Marques Neto, L. M., & Kipnis, A. (2014). Role of fused *Mycobacterium tuberculosis* immunogens and adjuvants in modern tuberculosis vaccines *Frontiers in Immunology*, *23*(5), 188–197

Kafri, M., Metzl-Raz, E., Jona, G., & Barkai, N. (2016). The cost of protein production. *Cell Reports*, *14*(1), 22–31.

Källberg, M., Wang, H., Wang, S., Peng, J., Wang, Z., Lu, H., & Xu, J. (2012). Template-based protein structure modeling using the RaptorX web server. *Nat. Protocols*, *7*(8), 1511–1522.

Kalyanasundram, J., Chia, S. L., Song, A. A.-L., Raha, A. R., Young, H. A., & Yusoff, K. (2015). Surface display of glycosylated Tyrosinase related protein-2 (TRP-2) tumour antigen on *Lactococcus lactis*. *BMC Biotechnology*, *15*, 113.

Kamath, A. T., Feng, C. G., Macdonald, M., Briscoe, H., & Britton, W. J. (1999). Differential protective efficacy of DNA vaccines expressing secreted proteins of *Mycobacterium tuberculosis*. *Infection and Immunity*, *67*(4), 1702–1707.

Kammona, O., & Kiparissides, C. (2012). Recent advances in nanocarrier-based mucosal delivery of biomolecules. *Journal of Controlled Release : Official Journal of the Controlled Release Society*, *161*(3), 781–94.

Kaufmann, S. H. E. (2013). Tuberculosis vaccines: Time to think about the next generation. *Seminars in Immunology*.

Kaushal, D., Mehra, S., Didier, P. J., & Lackner, A. A. (2012). The non-human primate model of tuberculosis. *Journal of Medical Primatology*, *41*(3), 191–201.

Keshavjee, S., & Farmer, P. E. (2012). Tuberculosis, drug resistance, and the history of modern medicine. *New England Journal of Medicine*, *367*(10), 931–936.

Klein, G., Pack, A., Bonaparte, C., & Reuter, G. (1998). Taxonomy and physiology of probiotic lactic acid bacteria. *International Journal of Food Microbiology*, *41*(2), 103–125.

König Helmut , Claus Harald, V. A. (2010). *Prokaryotic Cell Wall Compounds*. (H. König, C. Harald, & V. Ajit, Eds.) (1st ed.). Springer Berlin Heidelberg.

Kotton, C. N., & Hohmann, E. L. (2004). Enteric pathogens as vaccine vectors for foreign antigen selivery. *Infection and Immunity* , *72*(10), 5535–5547.

Kruisselbrink, A., Heijne Den Bak-Glashouwer, M.-J., Havenith, C. E. G., Thole, J. E. R., & Janssen, R. (2001). Recombinant *Lactobacillus plantarum* inhibits house dust mite-specific T-cell responses. *Clinical & Experimental Immunology*, *126*(1), 2–8.

Kuczkowska, K., Kleiveland, C. R., Minic, R., Moen, L. F., Øverland, L., Tjåland, R., Eijsink, V. G. H. (2017). Immunogenic Properties of *Lactobacillus plantarum* producing surface-displayed *Mycobacterium tuberculosis* antigens. *Applied and Environmental Microbiology* , *83*(2).

Kuipers, O. P., De Ruyter, P. G. G. A., Kleerebezem, M., & De Vos, W. M. (1998). Quorum sensing-controlled gene expression in lactic acid bacteria. *Journal of Biotechnology*, *64*(1), 15–21.

Kylä-Nikkilä, K., Alakuijala, U., & Saris, P. E. J. (2010). Immobilization of *Lactococcus lactis* to cellulosic material by cellulose-binding domain of Cellvibrio japonicus. *Journal of Applied Microbiology*, *109*(4), 1274–1283.

Langermans, J. a M., Doherty, T. M., Vervenne, R. W., van der Laan, T., Lyashchenko,

© COPYRIGHT UPM

K., Greenwald, R., Andersen, P. (2005). Protection of macaques against *Mycobacterium tuberculosis* infection by a subunit vaccine based on a fusion protein of antigen 85B and ESAT-6. *Vaccine*, *23*(21), 2740–50.

Lavelle, E. C., & Hagan, D. T. O. (2006). Delivery systems and adjuvants for oral vaccines. *Expert Opinion Drug Delivery,* 3(6): 747–762.

Lee, P. (2010). Biocontainment strategies for live lactic acid bacteria vaccine vectors. *Bioengineered Bugs*, *1*.

Leenhouts, K., Buist, G., & Kok, J. (1999). Anchoring of proteins to lactic acid bacteria. *Antonie van Leeuwenhoek*, *76*(1–4), 367–76.

Lefeber, D. J., Benaissa-Trouw, B., Vliegenthart, J. F. G., Kamerling, J. P., Jansen, W. T. M., Kraaijeveld, K., & Snippe, H. (2003). Th1-Directing adjuvants increase the immunogenicity of Oligosaccharide-Protein conjugate vaccines related to *Streptococcus pneumoniae* Type 3. *Infection and Immunity* , *71*(12), 6915–6920.

Lewinsohn, D. A., Swarbrick, G. M., Park, B., Cansler, M. E., Null, M. D., Toren, K. G., Lewinsohn, D. M. (2017). Comprehensive definition of human immunodominant CD8 antigens in tuberculosis. *Npj Vaccines*, *2*(1), 8.

Li, M. (2000). Applications of display technology in protein analysis. *Nature Biotechnology*, *18*(12), 1251–1256.

Li, W., Deng, G., Li, M., Zeng, J., Zhao, L., Liu, X., & Wang, Y. (2014). A recombinant adenovirus expressing CFP10, ESAT6, Ag85A and Ag85B of *Mycobacterium tuberculosis* elicits strong antigen-specific immune responses in mice. *Molecular Immunology*, *62*(1), 86–95.

Lin, C.-W., Su, I.-J., Chang, J.-R., Chen, Y.-Y., Lu, J.-J., & Dou, H.-Y. (2012). Recombinant BCG coexpressing Ag85B, CFP10, and interleukin-12 induces multifunctional Th1 and memory T cells in mice. *APMIS : Acta Pathologica, Microbiologica, et Immunologica Scandinavica*, *120*(1), 72–82.

Lin, P. L., Myers, A., Smith, L., Bigbee, C., Bigbee, M., Fuhrman, C., Flynn, J. L. (2010). Tumor necrosis factor neutralization results in disseminated disease in acute and latent *Mycobacterium tuberculosis* infection with normal granuloma structure in a cynomolgus macaque model. *Arthritis & Rheumatism*, *62*(2), 340–350.

Lindenstrøm, T., Agger, E. M., Korsholm, K. S., Darrah, P., Aagaard, C., Seder, R., Andersen, P. (2009). Tuberculosis subunit vaccination provides long-term protective immunity characterized by multifunctional CD4 memory T cells. *Journal of Immunology (Baltimore, Md. : 1950)*, *182*(12), 8047–55.

Liu, D., Wang, X., Ge, J., Liu, S., & Li, Y. (2011). Comparison of the immune responses induced by oral immunization of mice with *Lactobacillus casei*-expressing porcine parvovirus VP2 and VP2 fused to *Escherichia coli* heat-labile enterotoxin B. *"Comparative Immunology, Microbiology and Infectious Diseases," 34*(1), 73–81.

Liu, J., Tran, V., Leung, A. S., Alexander, D. C., & Zhu, B. (2009). BCG Vaccines: Their mechanisms of attenuation and impact on safety and protective efficacy. *Human Vaccines*, *5*(2), 70–78.

Luo, Y., Wang, B., Hu, L., Yu, H., Da, Z., Jiang, W., Zhu, B. (2009). Fusion protein Ag85B-MPT64(190-198)-Mtb8.4 has higher immunogenicity than Ag85B with capacity to boost BCG-primed immunity against *Mycobacterium tuberculosis* in mice. *Vaccine*, *27*(44), 6179–85.

Luz Correa, B., Ornaghi, A. P., Cerutti Muller, G., Engroff, P., Pestana Lopes, R., Gomes da Silva Filho, I., Bauer, M. E. (2014). The inverted CD4:CD8 ratio is associated with cytomegalovirus, poor cognitive and functional states in older adults. *Neuroimmunomodulation*, *21*(4), 206–212.

Lyadova, I. V, & Panteleev, A. V. (2015). Th1 and Th17 cells in tuberculosis: Protection,

© COPYRIGHT UPM

pathology, and biomarkers. *Mediators of Inflammation*, *2015*, 854507.
Ma, Y., Liu, J., Hou, J., Dong, Y., Lu, Y., Jin, L., Wu, J. (2014). Oral administration of recombinant *Lactococcus lactis* expressing HSP65 and tandemly repeated P277 reduces the incidence of type I diabetes in non-obese diabetic mice. *PLoS One*, *9*.
Macpherson, A. J., & Slack, E. (2007). The functional interactions of commensal bacteria with intestinal secretory IgA. *Current Opinion in Gastroenterology*, *23*(6).
Madhan Kumar, M., Raja, A., & Kumar, M. M. (2010). Cytotoxicity responses to selected ESAT-6 and CFP-10 peptides in tuberculosis. *Cellular Immunology*, *265*(2), 146–55.
Maidin, M. S. T., Song, A. A.-L., Jalilsood, T., Sieo, C. C., Yusoff, K., & Rahim, R. A. (2014). Construction of a novel inducible expression vector for *Lactococcus lactis* M4 and *Lactobacillus plantarum* Pa21. *Plasmid*, *74*, 32–8.
Majlessi, L., Simsova, M., Jarvis, Z., Brodin, P., Rojas, M.-J., Bauche, C., Leclerc, C. (2006). An increase in antimycobacterial Th1-cell responses by prime-boost protocols of immunization does not enhance protection against tuberculosis. *Infection and Immunity*, *74*(4), 2128–2137.
Malakar, P., & Venkatesh, K. V. (2012). Effect of substrate and IPTG concentrations on the burden to growth of *Escherichia coli* on glycerol due to the expression of Lac proteins. *Applied Microbiology and Biotechnology*, *93*(6), 2543–2549.
Marasini, N., Skwarczynski, M., & Toth, I. (2014). Oral delivery of nanoparticle-based vaccines. *Expert Review of Vaccines*, *13*(11), 1361–1376.
McShane, H., Pathan, A. A., Sander, C. R., Keating, S. M., Gilbert, S. C., Huygen, K., Hill, A. V. S. (2004). Recombinant modified vaccinia virus Ankara expressing antigen 85A boosts BCG-primed and naturally acquired antimycobacterial immunity in humans. *Nature Medicine*, *10*(11), 1240–1244.
Medina, E., & Guzmán, C. a. (2001). Use of live bacterial vaccine vectors for antigen delivery: potential and limitations. *Vaccine*, *19*(13–14), 1573–80.
Meena, L. S., & Rajni, (2010). Survival mechanisms of pathogenic *Mycobacterium tuberculosis* H37Rv. *FEBS Journal*, *277*(11), 2416–2427.
Méndez-Samperio, P. (2010). Role of interleukin-12 family cytokines in the cellular response to mycobacterial disease. *International Journal of Infectious Diseases*, *14*(5), e366–e371.
Mercenier, a, Müller-Alouf, H., & Grangette, C. (2000). Lactic acid bacteria as live vaccines. *Current Issues in Molecular Biology*, *2*(1), 17–25.
Mesnage, S., Dellarole, M., Baxter, N. J., Rouget, J.-B., Dimitrov, J. D., Wang, N., Williamson, M. P. (2014). Molecular basis for bacterial peptidoglycan recognition by LysM domains. *Nature communication*, 5, 4269.
Michon, C., Langella, P., Eijsink, V. G. H., Mathiesen, G., & Chatel, J. M. (2016). Display of recombinant proteins at the surface of lactic acid bacteria: strategies and applications. *Microbial Cell Factories*, *15*(1), 70.
Minassian, A. M., Ronan, E. O., Poyntz, H., Hill, A. V. S., & McShane, H. (2011). Preclinical development of an in vivo BCG challenge model for testing candidate TB vaccine efficacy. *PloS One*, *6*(5), e19840.
Minor, P. D. (2015). Live attenuated vaccines: Historical successes and current challenges. *Virology*, *479–480*, 379–392.
Mir, S. A., & Sharma, S. (2013). Cloning, expression and N-terminal formylation of ESAT-6 of *Mycobacterium tuberculosis* H37Rv. *Protein Expression and Purification*, *92*(2), 223–9.
Mitragotri, S. (2005). Immunization without needles, *Nature Reviews Immunology, 5* (December), 905–916.
Moeini, H., Rahim, R. A., Omar, A. R., Shafee, N., & Yusoff, K. (2011). *Lactobacillus*

acidophilus as a live vehicle for oral immunization against chicken anemia virus. *Applied Microbiology and Biotechnology*, *90*(1), 77–88.

Mohan, T., Verma, P., & Rao, D. N. (2013). Novel adjuvants & delivery vehicles for vaccines development: A road ahead. *The Indian Journal of Medical Research*, *138*(5), 779–795.

Mohan, V. P., Scanga, C. A., Yu, K., Scott, H. M., Tanaka, K. E., Tsang, E., Chan, J. (2001). Effects of tumor necrosis factor alpha on host immune response in chronic persistent tuberculosis: Possible role for limiting pathology. *Infection and Immunity*, *69*(3), 1847–1855.

Moise, L., Mcmurry, J., & Groot, A. S. De. (2009). *Mycobacterium tuberculosis*. *Vaccines for Biodefense and Emerging and Neglected Diseases,* 1, 1219–1239.

Moorthy, G., & Ramasamy, R. (2007). Mucosal immunisation of mice with malaria protein on lactic acid bacterial cell walls. *Vaccine*, *25*(18), 3636–45.

Morais Fonseca, D., Rosada, R. S., e Paula, M. O., Wowk, P. F., Franco, L. H., Soares, E. G., Deperon Bonato, V. L. (2010). Experimental tuberculosis: designing a better model to test vaccines against tuberculosis. *Tuberculosis (Edinburgh, Scotland)*, *90*(2), 135–42.

Moreno-Mendieta, S. A., Rocha-Zavaleta, L., & Rodriguez-Sanoja, R. (2010). Adjuvants in tuberculosis vaccine development. *FEMS Immunology & Medical Microbiology*, *58*(1), 75–84.

Nagy, G., Emo″dy, L., & Pál, T. (2008). Strategies for the development of vaccines conferring broad-spectrum protection. *International Journal of Medical Microbiology*, *298*(5–6), 379–395.

Niers, L. E. M., Timmerman, H. M., Rijkers, G. T., van Bleek, G. M., van Uden, N. O. P., Knol, E. F., Hoekstra, M. O. (2005). Identification of strong interleukin-10 inducing lactic acid bacteria which down-regulate T helper type 2 cytokines. *Clinical & Experimental Allergy*, *35*(11), 1481–1489.

Nochi, T., & Kiyono, H. (2006). Innate immunity in the mucosal immune system. *Current Pharmaceutical Desig*n,12, 4203–4213.

Norton, P. M., Wells, J. M., Brown, H. W., Macpherson, A. M., & Le Page, R. W. (1997). Protection against tetanus toxin in mice nasally immunized with recombinant *Lactococcus lactis* expressing tetanus toxin fragment C. *Vaccine*, *15*.

Oliveira, M. L. S., Arêas, A. P. M., Campos, I. B., Monedero, V., Perez-Martínez, G., Miyaji, E. N., Lee Ho, P. (2006). Induction of systemic and mucosal immune response and decrease in Streptococcus pneumoniae colonization by nasal inoculation of mice with recombinant lactic acid bacteria expressing pneumococcal surface antigen A. *Microbes and Infection / Institut Pasteur*, *8*(4), 1016–24.

Orme, I. M. (2003). The mouse as a useful model of tuberculosis. *Tuberculosis*, *83*(1–3), 112–115.

Orme, I. M. (2006). Preclinical testing of new vaccines for tuberculosis: A comprehensive review. *Vaccine*, *24*(1), 2–19.

Orme, I. M. (2014). A new unifying theory of the pathogenesis of tuberculosis. *Tuberculosis (Edinburgh, Scotland)*, *94*(1), 8–14.

Ou, C.-C., Lin, S.-L., Tsai, J.J., & Lin, M.-Y. (2011). Heat-killed lactic acid bacteria enhance immunomodulatory potential by skewing the immune response toward Th1 polarization. *Journal of Food Science*, *76*(5), M260-7.

Pareek, M., Greenaway, C., Noori, T., Munoz, J., & Zenner, D. (2016). The impact of migration on tuberculosis epidemiology and control in high-income countries: a review. *BMC Medicine*, *14*, 48.

Parida, S. K., & Kaufmann, S. H. E. (2010). Novel tuberculosis vaccines on the horizon.

© COPYRIGHT UPM

Current Opinion in Immunology, *22*(3), 374–84.
Pashine, A., Valiante, N. M., & Ulmer, J. B. (2005). Targeting the innate immune response with improved vaccine adjuvants. *Nature Medicine*, *11*(4), S63–S68.
Passot, S., Gautier, J., Jamme, F., Cenard, S., Dumas, P., & Fonseca, F. (2015). Understanding the cryotolerance of lactic acid bacteria using combined synchrotron infrared and fluorescence microscopies. *Analyst*, *140*(17), 5920–5928.
Pathan, A. A., Sander, C. R., Fletcher, H. A., Poulton, I., Alder, N. C., Beveridge, N. E. R., McShane, H. (2007). Boosting BCG with recombinant modified vaccinia ankara expressing antigen 85A: different boosting intervals and implications for efficacy trials. *PloS One*, *2*(10), e1052.
Pegg, D. E. (2015). Principles of cryopreservation. In J. G. Day & G. N. Stacey (Eds.), *Methods in Molecular Biology* (Vol. 1257, pp. 3–19). Totowa, NJ: Humana Press.
Perrie, Y., Mohammed, A. R., Kirby, D. J., McNeil, S. E., & Bramwell, V. W. (2008). Vaccine adjuvant systems: enhancing the efficacy of sub-unit protein antigens. *International Journal of Pharmaceutics*, *364*(2), 272–80.
Peternel, S., Grdadolnik, J., Gaberc-Porekar, V., & Komel, R. (2008). Engineering inclusion bodies for non denaturing extraction of functional proteins. *Microbial Cell Factories*, *7*, 34.
Pichichero, M. E. (2009). Booster Vaccinations: Can immunologic memory outpace disease pathogenesis? *Pediatrics*, *124*(6), 1633 LP-1641.
Pitt, J. M., Blankley, S., McShane, H., & O'Garra, A. (2013). Vaccination against tuberculosis: how can we better BCG? *Microbial Pathogenesis*, *58*, 2–16.
Piubelli, L., Campa, M., Temporini, C., Binda, E., Mangione, F., Amicosante, M., Pollegioni, L. (2013). Optimizing *Escherichia coli* as a protein expression platform to produce *Mycobacterium tuberculosis* immunogenic proteins. *Microbial Cell Factories*, *12*, 115.
Pochard, P., Gosset, P., Grangette, C., Andre, C., Tonnel, A.-B., Pestel, J., & Mercenier, A. (2017). Lactic acid bacteria inhibit T_H2 cytokine production by mononuclear cells from allergic patients. *Journal of Allergy and Clinical Immunology*, *110*(4), 617–623.
Pop, O. L., Diaconeasa, Z., Thorsten, B., Ciuzan, O., Pamfil, D., Vodnar, D. C., & Socaciu, C. (2015). Effect of glycerol, as cryoprotectant in the encapsulation and freeze drying of microspheres containing probiotic cells. *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Food Science and Technology*, *72*(1), 27–32.
Poquet , I. , Saint , V. , Seznec , E. , Simoes , N. , Bolotin , A. , and Gruss, A. (2000). HtrA is the unique surface housekeeping protease in *Lactococcus lactis* and is required for natural protein processing, *Molecular Microbiology*, *35*(5), 1042–1051.
Pouwels, P. H., Leer, R. J., Shaw, M., Bak-, M. H. Den, Tielen, F. D., Smit, E., Conway, P. L. (1998). Lactic acid bacteria as antigen delivery vehicles for oral immunization purposes. *International Journal of Food Microbiology*, *41*, 155–167.
Pydi, S. S., Bandaru, A. R., Venkatasubramanian, S., Jonnalagada, S., & Valluri, V. L. (2011). Vaccine for tuberculosis : Up-regulation of IL-15 by Ag85A and not by ESAT-6. *Tuberculosis*, *91*(2), 136–139.
Qiao, H., Duffy, L. C., Griffiths, E., Dryja, D., Leavens, A., Rossman, J., Locniskar, M. (2002). Immune responses in Rhesus Rotavirus-challenged Balb/c mice treated with Bifidobacteria and prebiotic supplements. *Pediatr Res*, *51*(6), 750–755.
Rafi, W., Ribeiro-Rodrigues, R., Ellner, J. J., & Salgame, P. (2012). "Coinfection-helminthes and tuberculosis". *Current Opinion in HIV and AIDS*, *7*(3), 239–244.

© COPYRIGHT UPM

Raha, A. R., Varma, N. R. S., Yusoff, K., Ross, E., & Foo, H. L. (2005). Cell surface display system for *Lactococcus lactis*: A novel development for oral vaccine. *Applied Microbiology and Biotechnology*, *68*(1), 75–81.

Rahman, S. (2013). *Local Immune Responses in Tuberculosis: Cytolytic Effector Functions at the Site of Mycobacterium tuberculosis Infection*. Karolinska institutet.

Ramasamy, R., Yasawardena, S., Zomer, A., Venema, G., Kok, J., & Leenhouts, K. (2006). Immunogenicity of a malaria parasite antigen displayed by *Lactococcus lactis* in oral immunisations. *Vaccine*, *24*(18), 3900–3908.

Reed, S. G., Coler, R. N., Dalemans, W., Tan, E. V, DeLa Cruz, E. C., Basaraba, R. J., Lobet, Y. (2009). Defined tuberculosis vaccine, Mtb72F/AS02A, evidence of protection in cynomolgus monkeys. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(7), 2301–2306.

Repa, A., Grangette, C., Daniel, C., Hochreiter, R., Hoffmann-sommergruber, K., Thalhamer, J., Wiedermann, U. (2003). Mucosal co-application of lactic acid bacteria and allergen induces counter-regulatory immune responses in a murine model of birch pollen allergy, *22*, 87–95.

Ribelles, P., Benbouziane, B., Langella, P., Suárez, J. E., Bermúdez-Humarán, L. G., & Riazi, A. (2013). Protection against human papillomavirus type 16-induced tumors in mice using non-genetically modified lactic acid bacteria displaying E7 antigen at its surface. *Applied Microbiology and Biotechnology*, *97*(3), 1231–1239.

Robinson, K., Chamberlain, L. M., Schofield, K. M., Wells, J. M., & Le Page, R. W. (1997). Oral vaccination of mice against tetanus with recombinant *Lactococcus lactis*. *Nat Biotechnol*, *15*.

Rodriguez, J. M., Cintas, L. M., Casaus, P., Horn, N., Dodd, H. M., Hernandez, P. E., & Gasson, M. J. (1995). Isolation of nisin-producing *Lactococcus lactis* strains from dry fermented sausages. *The Journal of Applied Bacteriology*, *78*(2), 109–115.

Rosada, R. S., de la Torre, L. G., Frantz, F. G., Trombone, A. P. F., Zárate-Bladés, C. R., Fonseca, D. M., Coelho-Castelo, A. a M. (2008). Protection against tuberculosis by a single intranasal administration of DNA-hsp65 vaccine complexed with cationic liposomes. *BMC Immunology*, *9*, 38.

Russell, D. G. (2001). *Mycobacterium tuberculosis*: here today, and here tomorrow. *Nature Reviews. Molecular Cell Biology*, *2*(8), 569–577.

Sable, S. B., Verma, I., & Khuller, G. K. (2005). Multicomponent antituberculous subunit vaccine based on immunodominant antigens of *Mycobacterium tuberculosis*. *Vaccine*, *23*(32), 4175–84.

Sáez, D., Fernández, P., Rivera, A., Andrews, E., & Oñate, A. (2012). Oral immunization of mice with recombinant *Lactococcus lactis* expressing Cu,Zn superoxide dismutase of Brucella abortus triggers protective immunity. *Vaccine*, *30*(7), 1283–90.

Sakamoto, K. (2012). The Pathology of *Mycobacterium tuberculosis* Infection. *Veterinary Pathology*, *49*(3), 423–439.

Salgame, P., Yap, G. S., & Gause, W. C. (2013). Effect of helminth-induced immunity on infections with microbial pathogens. *Nature Immunology*, *14*(11), 1118–1126.

Sambrook, J., & Russel, D. W. (2001). *Molecular Cloning: A Laboratory Manual, 3rd edn.* (3rd ed.). Cold Spring Harbor Laboratory Press.

Samuelson, P., Gunneriusson, E., Nygren, P. A., & Ståhl, S. (2002). Display of proteins on bacteria. *Journal of Biotechnology*, *96*(2), 129–54.

Sarhan, M. a a, Musa, M., & Zainuddin, Z. F. (2011). Cloning, protein expression and display of synthetic multi-epitope mycobacterial antigens on Salmonella typhi Ty21a cell surface. *Indian Journal of Experimental Biology*, *49*(9), 645–53.

© COPYRIGHT UPM

Sawamiphak, S., Kontarakis, Z., & Stainier, D. Y. R. (2014). Interferon gamma signaling positively regulates hematopoietic stem cell emergence. *Developmental Cell*, *31*(5), 640–653.

Scaldaferri, F., Gerardi, V., Lopetuso, L. R., Del Zompo, F., Mangiola, F., Boškoski, I., Gasbarrini, A. (2013). Gut microbial flora, prebiotics, and probiotics in IBD: Their current usage and utility. *BioMed Research International*, 3, 55-64.

Seegers, J. F. M. L. (2002). Lactobacilli as live vaccine delivery vectors : progress and prospects. *Trends in Biotechnology*,*20*(12), 508–515.

Sherman, D. R., Guinn, K. M., Hickey, M. J., Mathur, S. K., Zakel, K. L., & Smith, S. (2004). *Mycobacterium tuberculosis* H37Rv: Delta RD1 is more virulent than *M. bovi*s bacille Calmette-Guerin in long-term murine infection. *The Journal of Infectious Diseases*, *190*(1), 123–126.

Shi, S., Yu, L., Sun, D., Liu, J., & Hickey, A. J. (2010). Rational design of multiple TB antigens TB104 and TB104-Ag85B as subunit vaccine candidates against *Mycobacterium tuberculosis*, *27*(2), 224–235.

Şimşek, Ö., Sabanoğlu, S., Çon, A. H., Karasu, N., Akçelik, M., & Saris, P. E. J. (2013). Immobilization of nisin producer *Lactococcus lactis* strains to chitin with surface-displayed chitin-binding domain. *Applied Microbiology and Biotechnology*, *97*(10), 4577–4587.

Singh, S. M., & Panda, A. K. (2005). Solubilization and refolding of bacterial inclusion body proteins. *Journal of Bioscience and Bioengineering*, *99*(4), 303–10.

Skeiky, Y. A. W., Alderson, M. R., Ovendale, P. J., Guderian, J. A., Brandt, L., Dillon, D. C., Reed, S. G. (2004). Differential immune responses and protective efficacy induced by components of a tuberculosis polyprotein vaccine, Mtb72F, delivered as naked DNA or recombinant protein. *Journal of Immunology (Baltimore, Md. : 1950)*, *172*(12), 7618–7628.

Slight, S. R., & Khader, S. a. (2013). Chemokines shape the immune responses to tuberculosis. *Cytokine & Growth Factor Reviews*, *24*(2), 105–13.

Smelt, M. J., de Haan, B. J., Bron, P. a, van Swam, I., Meijerink, M., Wells, J. M., de Vos, P. (2012). L. plantarum, L. salivarius, and *L. lactis* attenuate Th2 responses and increase Treg frequencies in healthy mice in a strain dependent manner. *PloS One*, *7*(10), e47244.

Smelt, M. J., Haan, B. J. De, Bron, P. A., Swam, I. Van, Meijerink, M., Wells, J. M., Vos, P. De. (2012). Responses and increase Treg frequencies in healthy mice in a strain dependent manner, *7*(10), 1–14.

Smith, D. W., & Harding, G. E. (1977). Animal model of human disease. Pulmonary tuberculosis. Animal model: Experimental airborne tuberculosis in the guinea pig. *The American Journal of Pathology*, *89*(1), 273–276.

Smith, D., Wiegeshaus, E., & Balasubramanian, V. (2000). Animal models for experimental tuberculosis. *Clinical Infectious Diseases* , *31*(Supplement 3), S68–S70.

Smith, I. (2003). *Mycobacterium tuberculosis* pathogenesis and molecular determinants of virulence. *Clinical Microbiology Reviews*, *16*(3), 463–496.

Songane, M., Kleinnijenhuis, J., Netea, M. G., & van Crevel, R. (2012). The role of autophagy in host defence against *Mycobacterium tuberculosis* infection. *Tuberculosis (Edinburgh, Scotland)*, *92*(5), 388–96.

Spertini, F., Audran, R., Lurati, F., Ofori-Anyinam, O., Zysset, F., Vandepapeliere, P., Ballou, W. R. (2013). The candidate tuberculosis vaccine Mtb72F/AS02 in PPD positive adults: a randomized controlled phase I/II study. *Tuberculosis (Edinburgh, Scotland)*, *93*(2), 179–188.

Staats, H. F. (1996). Application of basic principles of mucosal immunity to vaccine

© COPYRIGHT UPM

development. In Hiroshi Kiyono, P. Ogra, & J. McGhee (Eds.), *Mucosal Vaccines* (1st ed., pp. 17–39). San Diego: Academic Press.

Stackebrandt, E., Teuber, M., Mikrobiologie, A., Universit, D., Mikrobiologie, I., Milchforschung, D. B., & Str, H. W. (1988). Molecular taxonomy and phylogenetic position of lactic acid bacteria, *70*, 317–324.

Steen, A., Buist, G., Horsburgh, G. J., Venema, G., Kuipers, O. P., Foster, S. J., & Kok, J. (2005). AcmA of *Lactococcus lactis* is an N-acetylglucosaminidase with an optimal number of LysM domains for proper functioning. *The FEBS Journal*, *272*(11), 2854–68.

Steen, A., Buist, G., Leenhouts, K. J., El Khattabi, M., Grijpstra, F., Zomer, A. L., Kok, J. (2003). Cell wall attachment of a widely distributed peptidoglycan binding domain is hindered by cell wall constituents. *The Journal of Biological Chemistry*, *278*(26), 23874–81.

Steen, A., Steen, A., Buist, G., Buist, G., Kok, J., Kok, J., Leenhouts, K. (2006). Novel Surface Display system for proteins on non-genetically Modified Gram-Positive bacteria. *Society*, *72*(1), 880–889.

Steidler, L., Robinson, K., Chamberlain, L., Schofield, K. M., Remaut, E., Le Page, R. W., & Wells, J. M. (1998). Mucosal delivery of murine interleukin-2 (IL-2) and IL-6 by recombinant strains of *Lactococcus lactis* coexpressing antigen and cytokine. *Infect Immun*, *66*.

Storgaard, M., Varming, K., Herlin, T., & Obel, N. (2006). Novel mutation in the interferon-gamma-receptor gene and susceptibility to mycobacterial infections. *Scandinavian Journal of Immunology*, *64*(2), 137–139.

Tao, H., Liu, W., Simmons, B. N., Harris, H. K., Cox, T. C., & Massiah, M. a. (2010). Purifying natively folded proteins from inclusion bodies using sarkosyl, Triton X-100, and CHAPS. *BioTechniques*, *48*(1), 61–4.

Tarahomjoo, S. (2013). Exploring surface display technology for enhancement of delivering viable lactic acid bacteria to gastrointestinal tract. Kongo Health and Livestock Purposes (Ed.), *Lactic Acid Bacteria - R & D for Food, Health and Livestock Purposes* (pp. 427–454). Rijeka: InTech.

Terzaghi, B. E., & Sandine, W. E. (1975). Improved medium for lactic Streptococci and their Bacteriophages. *Applied Microbiology*, *29*(6), 807–813.

Tsolaki, A. G., Nagy, J., Leiva, S., Kishore, U., Rosenkrands, I., & Robertson, B. D. (2013). *Mycobacterium tuberculosis* antigen 85B and ESAT-6 expressed as a recombinant fusion protein in *Mycobacterium smegmatis* elicits cell-mediated immune response in a murine vaccination model. *Molecular Immunology*, *54*(3–4), 278–83.

van Asseldonk, M., de Vos, W. M., & Simons, G. (1993). Functional analysis of the *Lactococcus lactis* usp45 secretion signal in the secretion of a homologous proteinase and a heterologous alpha-amylase. *Molecular and General Genetic*, *240*(3)*,* 428-434

van Crevel, R., Karyadi, E., Preyers, F., Leenders, M., Kullberg, B.-J., Nelwan, R. H. H., & van der Meer, J. W. M. (2000). Increased production of Interleukin 4 by CD4+ and CD8+ T cells from patients with tuberculosis is related to the presence of pulmonary cavities. *Journal of Infectious Diseases* , *181*(3), 1194–1197.

van Crevel, R., Ottenhoff, T. H. M., & van der Meer, J. W. M. (2002). Innate immunity to *Mycobacterium tuberculosis*. *Clinical Microbiology Reviews*, *15*(2), 294–309.

van der Wel, N., Hava, D., Houben, D., Fluitsma, D., van Zon, M., Pierson, J., Peters, P. J. (2007). *M. tuberculosis* and *M. leprae* translocate from the phagolysosome to the cytosol in myeloid cells. *Cell*, *129*(7), 1287–1298.

Van Deun, A., Hossain, M. A., Gumusboga, M., & Rieder, H. L. (2008). Ziehl-Neelsen

© COPYRIGHT UPM

staining: theory and practice. *The International Journal of Tuberculosis and Lung Disease : The Official Journal of the International Union against Tuberculosis and Lung Disease*, *12*(1), 108–110.
van Dissel, J. T., Soonawala, D., Joosten, S. A., Prins, C., Arend, S. M., Bang, P., Andersen, P. (2011, March 3). Ag85B-ESAT-6 adjuvanted with IC31 promotes strong and long-lived *Mycobacterium tuberculosis* specific T cell responses in volunteers with previous BCG vaccination or tuberculosis infection. *Vaccine*, *3;29*(11), 2100-2109.
Van Roosmalen, M. L., Kanninga, R., El Khattabi, M., Neef, J., Audouy, S., Bosma, T., Leenhouts, K. (2006). Mucosal vaccine delivery of antigens tightly bound to an adjuvant particle made from food-grade bacteria. *Methods*, *38*(2), 144–149.
Vandamme, P., De Bruyne, K., & Pot, B. (2014). Phylogenetics and systematics. In *Lactic Acid Bacteria: Biodiversity and Taxonomy* (pp. 31–44). John Wiley & Sons, Ltd.
Vandenbroucke, K., Haard, H., Beirnaert, E., Dreier, T., Lauwereys, M., Huyck, L., Remaut, E. (2010). Orally administered *L. lactis* secreting an anti-TNF nanobody demonstrate efficacy in chronic colitis. *Mucosal Immunollogy*, *3*(1), 49-56
Varma, N. R. S., Toosa, H., Foo, H. L., Alitheen, N. B. M., Nor Shamsudin, M., Arbab, A. S., Abdul Rahim, R. (2013). Display of the Viral Epitopes on *Lactococcus lactis*: A model for food grade vaccine against EV71. *Biotechnology Research International*, *2013*, 431315.
Velayati, A. A., & Farnia, P. (2012). Morphological characterization of *Mycobacterium tuberculosis*. *Understanding Tuberculosis - Deciphering the Secret Life of the Bacilli*, (January), 149–167.
Vidarsson, G., Dekkers, G., & Rispens, T. (2014). IgG subclasses and allotypes: From structure to effector functions. *Frontiers in Immunology*, *5*, 520.
Visweswaran, G. R. R., Leenhouts, K., Van Roosmalen, M., Kok, J., & Buist, G. (2014). Exploiting the peptidoglycan-binding motif, LysM, for medical and industrial applications. *Applied Microbiology and Biotechnology*, *98*(10), 4331–4345.
Visweswaran, G. R. R., Steen, A., Leenhouts, K., Szeliga, M., Ruban, B., Hesseling-Meinders, A., Buist, G. (2013). AcmD, a homolog of the major autolysin AcmA of *Lactococcus lactis*, binds to the cell wall and contributes to cell separation and autolysis. *PLoS ONE*, *8*(8), 1–11.
Von Eschen, K., Morrison, R., Braun, M., Ofori-Anyinam, O., De Kock, E., Pavithran, P., Ballou, W. R. (2009). The candidate tuberculosis vaccine Mtb72F/AS02A: Tolerability and immunogenicity in humans. *Human Vaccines*, *5*(7), 475–482.
Walker, K. B., Brennan, M. J., Ho, M. M., Eskola, J., Thiry, G., Sadoff, J., Lambert, P. H. (2010). The second Geneva Consensus: Recommendations for novel live TB vaccines. *Vaccine*, *28*(11), 2259–70.
Wang, S., Liu, H., Zhang, X., & Qian, F. (2015). Intranasal and oral vaccination with protein-based antigens: advantages, challenges and formulation strategies. *Protein & Cell*, *6*(7), 480–503.
Wang, W., Nema, S., & Teagarden, D. (2010). Protein aggregation-Pathways and influencing factors. *International Journal of Pharmaceutics*, *390*(2), 89–99.
Wang, Z., Zhao, F., Peng, J., & Xu, J. (2011). Protein 8-class secondary structure prediction using conditional neural fields. *Proteomics*, *11*(19), 3786–3792.
Warrell, D. A., & Madkour, M. M. (2011). *Tuberculosis*. (M. M. Madkour, Ed.) (illustrate). Springer Berlin Heidelberg.
Wells, J. M., & Mercenier, A. (2008). Mucosal delivery of therapeutic and prophylactic molecules using lactic acid bacteria. *Nature Reviews. Microbiology*, *6*(5), 349–62.
WHO. (2014). Global strategy and targets for tuberculosis prevention, care and control

© COPYRIGHT UPM

after 2015. *Geneva: World Health Organization*, 2014–2015.
WHO. (2015). Global Tuberculosis Report 2015. *World Health Organization*, *53*, 160.
Wieczorek, A. E. (2011). *Characterizing the role of the hspx protein from Mycobacterium tuberculosis as a subunit vaccine candidate in the small animal model of tuberculosis*. Colorado State University.
Windish, H. P., Duthie, M. S., Misquith, A., Ireton, G., Lucas, E., Laurance, J. D., Reed, S. G. (2011). Protection of mice from *Mycobacterium tuberculosis* by ID87/GLA-SE, a novel tuberculosis subunit vaccine candidate. *Vaccine*, *29*(44), 7842–8.
Wong, J. E. M. M., Alsarraf, H. M. A. B., Kaspersen, J. D., Pedersen, J. S., Stougaard, J., Thirup, S., & Blaise, M. (2014). Cooperative binding of LysM domains determines the carbohydrate affinity of a bacterial endopeptidase protein. *FEBS Journal*, *281*(4), 1196–1208.
Woof, J. M., & Kerr, M. A. (2006). The function of immunoglobulin A in immunity. *The Journal of Pathology*, *208*(2), 270–282.
Wu, C., Yang, G., Bermúdez-Humarán, L. G., Pang, Q., Zeng, Y., Wang, J., & Gao, X. (2006). Immunomodulatory effects of IL-12 secreted by *Lactococcus lactis* on Th1/Th2 balance in ovalbumin (OVA)-induced asthma model mice. *International Immunopharmacology*, *6*(4), 610–615.
Xie, X., Han, M., Zhang, L., Liu, L., Gu, Z., Yang, M., & Yang, H. (2016). Effects of *Mycobacterium tuberculosis* ESAT6-CFP10 protein on cell viability and production of nitric oxide in alveolar macrophages. *Jundishapur J Microbiol*, *9*(6).
Xie, X., Li, F., Chen, J.-W., & Wang, J. (2014). Risk of tuberculosis infection in anti-TNF-α biological therapy: From bench to bedside. *Journal of Microbiology, Immunology and Infection*, *47*(4), 268–274.
Xin, Q., Niu, H., Li, Z., Zhang, G., Hu, L., Wang, B., Zhu, B. (2013). Subunit vaccine consisting of multi-stage antigens has high protective efficacy against *Mycobacterium tuberculosis* infection in mice. *PloS One*, *8*(8), e72745.
Xing, Z., & Lichty, B. D. (2006). Use of recombinant virus-vectored tuberculosis vaccines for respiratory mucosal immunization. *Tuberculosis (Edinburgh, Scotland)*, *86*(3–4), 211–7.
Yang, X.-Y., Chen, Q.F., Li, Y.P., & Wu, S.-M. (2011). *Mycobacterium vaccae* as adjuvant therapy to anti-tuberculosis chemotherapy in never-treated tuberculosis patients: A Meta-analysis. *PLoS ONE*, *6*(9), e23826.
Young, D. B., Perkins, M. D., Duncan, K., & Barry, C. E. (2008). Confronting the scientific obstacles to global control of tuberculosis. *The Journal of Clinical Investigation*, *118*(4), 1255–1265.
Yuan, W., Dong, N., Zhang, L., Liu, J., Lin, S., Xiang, Z., Qin, C. (2012, March 23). Immunogenicity and protective efficacy of a tuberculosis DNA vaccine expressing a fusion protein of Ag85B-Esat6-HspX in mice. *Vaccine*, 30(14), 2490-2497.
Zou, Y., Mi, J., Wang, W., Lu, J., Zhao, W., Liu, Z., Gong, Y. (2013). CUL4B promotes replication licensing by up-regulating the CDK2-CDC6 cascade. *The Journal of Cell Biology*, *200*(6), 743–56.
Zumla, A. I., Gillespie, S. H., Hoelscher, M., Philips, P. P. J., Cole, S. T., Abubakar, I., Nunn, A. J. (2014). New antituberculosis drugs, regimens, and adjunct therapies: needs, advances, and future prospects. *The Lancet Infectious Diseases*, *14*(4), 327–340.

© COPYRIGHT UPM