UNIVERSITI PUTRA MALAYSIA

CONSTRUCTION AND CHARACTERIZATION OF A LACTOCOCCUS LACTIS IN-TRANS SURFACE DISPLAY SYSTEM HARBORING MURINE GLYCOSYLATED TYROSINASE RELATED PROTEIN-2

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

CONSTRUCTION AND CHARACTERIZATION OF A *LACTOCOCCUS LACTIS IN-TRANS* SURFACE DISPLAY SYSTEM HARBORING MURINE GLYCOSYLATED TYROSINASE RELATED PROTEIN-2

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Food and commensal lactic acid bacteria (LAB) surface display system exploitation for bacterial, viral, or protozoal antigen delivery has received immense interest currently. The Generally Regarded as Safe (GRAS) status of LAB such as *Lactococcus lactis* coupled with non-recombinant strategy of *in-trans* surface display system, provide a safe platform for therapeutic drug and vaccine development. However, therapeutic proteins fused with cell-wall anchoring motif production are predominantly limited to prokaryotic expression system. This presents a major disadvantage in surface display system particularly when glycosylation has been recently identified to significantly enhance epitope presentation. In this study, glycosylated murine Tyrosinase related protein-2, mTRP-2, tumor associated antigen anchoring to *L. lactis* cell wall was attempted. The *mtrp-2-cA* (AcmA, peptidoglycan anchoring motif) fusion gene expression in Chinese Hamster Ovary, CHO cells was carried out. Initial CHO cell expression of both native *mtrp-2* and *mtrp-cA* was a failure. Codon optimized *mtrp-2* and *cA* genes also did not result in target protein production. In order to investigate post-translational modification interruption, expression of codon optimized *mtrp-2*$_{125\text{-}276}$ epitope devoid of *mtrp-2* native maturation signal peptide was performed which resulted in misfolded plus aggregated mTRP-2$_{125\text{-}276}$ and mTRP-2$_{125\text{-}276}$ –cA protein production. Successful expression of both *mtrp-2*$_{125\text{-}276}$ and *mtrp-2*$_{125\text{-}276}$ *-cA* genes suggest CHO cell's endoplasmic reticulum signal peptidase inability to recognize mTRP-2 signal peptide cleavage site. The following substitution of native mTRP-2 signal peptide with Chinese Hamster TRP-2 signal peptide, CHsp resolved this issue by successful expression of soluble mTRP-2 and mTRP-2-cA by CHO cells in both intracellular and extracellular fraction. A total amount of 40 µg of mTRP-2-cA protein from 2.7 g in wet weight of CHO cells was purified and detected to be glycosylated by glycoprotein staining. Subsequent mTRP-2-cA anchoring to the cell wall of *L. lactis* showed excitation of FITC conjugate on secondary antibody which signified successful binding of glycosylated TRP-2 on the surface of *L. lactis*.

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Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Sarjana Sains

PEMBINAAN DAN PECIRIAN SISTEM PEMPAMERAN PERMUKAAN *IN-TRANS LACTOCOCCUS LACTIS* YANG MEMBAWA PROTEIN BERKAITAN TIROSINASE-2 TIKUS TERGLIKOSILAT

Oleh

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Sejak kebelakangan ini, eksploitasi sistem paparan permukaan bakteria makanan dan komensal seperti Bakteria Laktik Asid (LAB) untuk penyajian antigen bakteria, virus dan protozoa semakin menerima perhatian. Status LAB yang secara umumnya dianggap selamat, (GRAS) seperti *Lactococcus lactis* dan strategi bukan rekombinasi sistem paparan permukaan *in-trans*, mewujudkan satu platform yang selamat bagi penghasilan dadah dan vaksin terapeutik. Walau bagaimanapun, strategi penggabungan protein terapeutik dengan motif pautan dinding sel kebanyakannya terhad kepada sistem pengekspresan prokariot. Ini merupakan satu kelemahan sistem paparan permukaan bakteria terutamanya apabila glikolisasi baru-baru ini dikenal pasti meningkatkan kebolehan pempameran epitop. Dalam penyelidikan ini, pautan antigen berkaitan dengan tumor, *Protein berkaitan Tirosinase-2*, tikus, mTRP-2 yang terglikosilat, pada dinding sel *L. lactis* dikaji. Pengekspresan gen gabungan *mtrp-2-cA* (motif pautan peptidoglikan AcmA) dalam sel Ovari Hamster Cina, CHO dijalankan. Pada permulaannya, sel CHO gagal mengekspres gen *mtrp-2* dan *mtrp-2-cA* yang asli. Pengoptimuman kodon bagi gen *mtrp-2* dan *cA* juga tidak menghasilkan protein sasaran. Bagi menyiasat gangguan modifikasi pasca translasi, pengekspresan epitop *mtrp-2*$_{125\text{-}276}$ yang tidak mempunyai peptida isyarat kematangan asli, dijalankan. Strategi ini menghasilkan protein mTRP-2$_{125\text{-}276}$ dan mTRP-2$_{125\text{-}276}$ −cA yang tergumpal dan tersalah lipat. Kejayaan pengekspresan gen *mtrp-2*$_{125\text{-}276}$ dan *mtrp-2*$_{125\text{-}276}$*-cA* mencadangkan kegagalan enzim peptidase isyarat retikulum endoplasma sel CHO mengenal pasti tapak pemotongan peptida isyarat. Sehubungan dengan itu, penukaran peptida isyarat mTRP-2 asli kepada peptida isyarat TRP-2 Hamster Cina, CHsp dijalankan. Strategi ini berjaya menyelesaikan masalah pengekspresan gen melalui penghasilan mTRP-2 and mTRP-2-cA yang terlarut oleh sel CHO dalam bahagian intrasel dan ekstrasel. Sejumlah 40 µg mTRP-2-cA protein daripada berat basah 2.7 g sel CHO telah berjaya ditulenkan dan hasil glikosilasi dikesan melalui kaedah pewarnaan glikoprotein. Ini dikuti dengan analisa pautan mTRP-2-cA pada dinding sel *L. lactis* yang menunjukkan pengujaan konjugat FITC pada antibodi sekunder, menandakan kejayaan pautan TRP-2 terglikosilat pada permukaan *L. lactis.*

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APPROVAL

I certify that a Thesis Examination Committee has met on 4th February 2015 to conduct the final examination of Jeevanathan Kalyanasundram on his thesis entitled "Construction and Characterization of a *Lactococcus lactis in-trans* Surface Display System Harboring Murine Glycosylated Tyrosinase Related Protein-2" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the University Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be awarded the degree of Master of Science.

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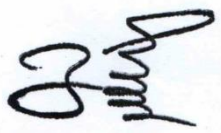

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

~	approximately
°C	degree Celcius
µg	microgram
µl	microlitre
AcmA	N-acetylglucosamidase
BLAST	Basic Local Alignment Search Tool
BSA	Bovine Serum Albumin
bp	base pairs
$CaCl_2$	calcium chloride
cDNA	complementary deoxynucleotide acid
CHO	Chinese Hamster Ovary
CWBD	Choline binding domain
Da	Dalton
DCT	DOPAchrome tautomerase
dH_20	distilled water
DHI	5,6-dihydroxyindole
DHICA	5,6-dihydroxyindole-2-carboxylic acid
DNA	deoxyribonucleotide acid
dNTP	deoxyribonucleotide triphosphate
DOPA	L-3,4 –dihydroxyphenylalanine
DQ	DOPAquinone
EDTA	Ethylenediaminetetraacetic acid
EJC	Exon junction complex
ER	Endoplasmic reticulum
eV	electron volt
g	gravity force
GAD	glutamate decarboxylase
GEM	Gram-positive Enhancer Matrix
GlcNAc	N-acetyl-D-glucosamine
GM17	M17 supplemented with 0.5% glucose
GRAS	Generally Regarded as Safe
h	hour
HIF	hypoxia inducible factors
HRP	Horse Radish Peroxidase
hRNP	heteronuclear ribonuclear protein
kb	kilo base pairs
kDa	kilo Dalton
kV	kiloVolt
l	litre
LAB	Lactic Acid Bacteria
LB	Luria-Bertani
LysM	Lysin Motif
M	Molar
mA	milliampere
MBP	Membrane Bound Protein

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min	minute
mg	milligram
ml	millilitre
mm	millimetre
mM	millimolar
$MgCl_2$	magnesium chloride
mRNA	messager Ribonucleic acid
MurNAc	N-acetylmuramic acid
NaCl	sodium chloride
NaOH	sodium hydroxide
NCBI	National Center for Biotechnology Information
ng	nanogram
NICE	Nisin Controlled Gene Expression
OD	Optical Density
PCR	Polymerase Chain Reaction
PTM	Post Translational Modifications
RE	Restriction enzymes
RNAPII	RNA Polymerase II
rpm	revolutions per minute
RT	retention time
SAm	Surface Anchoring motif
SCWP	Secondary cell wall polymers
SDS-PAGE	Sodium dodecyl sulfate-Polyacrylamide gel electrophoresis
sec	seconds
SLHD	S-layer Homology Domain
SRP	Signal Recognition Particle
TAA	Tumour associated antigen
Ta	annealing temperature
TBP	TATA-binding protein
TCA	Trichloroacetic acid
TF	Transcription Factor
TGN	Trans-golgi network
Tm	melting temperature
TRP-1	Tyrosinase-related protein 1
TRP-2	Tyrosinase-related protein 2
TSA	Tumour specific antigen
V	volt
v/v	volume per volume
VTC	vasicular tubular complexes
W	Watts
w/v	weight per volum

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CHAPTER 1

INTRODUCTION

The utilization of food and commensal lactic acid bacteria (LAB) as cellular vehicles for vaccine delivery has received immense interest over the past decade. Besides their GRAS (generally regarded as safe) status compared to their attenuated pathogenic counterparts, the LAB have the ability to colonize animal and human gastrointestinal tracts or genital mucosa with probiotic and immunomodulatory properties. This has made LAB an excellent candidate for oral and intranasal vaccine development (Pontes *et al.,* 2011; Raha *et al.,* 2005). Therefore, the *Lacotococcus lactis* can be genetically engineered to become an efficient recombinant cell factory for DNA delivery as well as production and presentation of antigens (Pontes *et al.,* 2011; Morello *et al.,* 2008). Such presentation of antigens through surface display or secretion by *L. lactis* in numerous studies utilizes the well understood and characterized surface binding protein domain such as transmembrane domains, lysin M, LysM and LPXTG motifs (Bahey-El-Din *et al.,* 2010; Raha *et al.,* 2005).

Based on the above, the LAB has the potential to be developed as a tumour antigen carrier for therapeutic or prophylactic cancer vaccines. Such cancer vaccines would able to mount sustainable immune response to eradicate primary tumour as well as prevent cancer relapses (Pejawar-Gaddy and Finn, 2008). Nevertheless, despite the early discovery of probiotic antitumour activity (Kelkar *et al.,* 1988), the utilization of LAB in anticancer therapy has been limited to cytotoxicity reduction of drugs used in chemotherapy and radiation therapy (Mego *et al.,* 2005). A part from that, the LAB has only been manipulated as prophylactic adjuvants in the prevention of colorectal cancer (Satonaka *et al.,* 1996). Cancer antigen delivery by the LAB, on the other hand, has not been widely explored and was only limited to surface displaying viral antigens from the human papillomavirus type-16 (HPV-16) E7 antigen on *L. lactis* , *Lactobacillus plantarum* and *Lactobacillus casei* for cervical cancer treatment (Ribelles *et al.,* 2013; Cortes-Perez *et al.,* 2005).

The TRP-2 (Tyrosinase related protein-2) is a tumor-associated antigen involved in melanin biosynthesis of both melanocytes and melanoma. TRP-2 has also been intensely studied as a viable therapeutic and prophylactic vaccine candidate for melanoma and glioblastoma (Yamano *et al.,* 2005; InSug *et al.,* 2003). The TRP-2 peptide vaccination alone only resulted in weak T cell response with insignificant tumouricidal effect (Jia *et al.,* 2005). Subsequent attempts to improve the TRP-2 immunogenecity and antigen presentations through plasmid DNA vaccination has been relatively inefficient in inducing antibody response and cellular mediated immunity toward TRP-2 (Yamano *et al.,* 2005). Nevertheless, the TRP-2 DNA vaccination for glioblastomamultiforme treatment has resulted in tumour regression and immunological targeting to increase chemotherapeutic drugs sensitivity (Liu *et al.,* 2005; InSug *et al.,* 2003). Therapeutic effects for melanoma by alphavirus replicon (Avogadri *et al.,* 2010), cytomegalovirus (CMV) (Xu *et al.,* 2013), attenuated *Salmonella typhimurium* (Zhu *et al.,* 2010) and *Listeria Monocytogenes* (Bruhn *et al.,* 2005) carrying TRP-2 have also been reported. Surprisingly, despite well documented adjuvancy of LABs in mucosal immunogenicity (Kajikawa *et al.,* 2010; Mercenier *et al.,* 2000), these GRAS status bacteria have yet to be manipulated to introduce TRP-2 gene for both therapeutic and prophylactic settings. In addition,

common autoimmunity side effect of hypopigmentation (vitiligo) resulting from TRP-2 (self-antigen) immunization have been observed to be dependent on the vaccine strategies (Avogadri *et al.*, 2010; Steitz *et al.*, 2000) suggesting the unknown possibility of GRAS bacteria carrying TRP-2 in generating autoreactive T-cells.

In this study, construction of live *L. lactis* surface displaying TRP-2 was attempted. The novel concept of introducing post-translationally modified TRP-2, *in-trans* to *L. lactis* peptidoglycan was explored. The prospect of using non-recombinant prokaryotes to deliver glycosylated eukaryotic protein, particularly in vaccine application is an attractive one. Recently, N-glycosylation has been identified to significantly enhance epitope presentation of MHC class I molecules by using tyrosinase as model antigen (Ostankovitch *et al.*, 2009). However, the surface display strategy for glycosylated proteins has been restricted to the yeast system which has been a key advantage over other surface display strategies (Boder *et al.*, 1997). Despite such advantage, different linkage of carbohydrate moieties (primarily mannose) to the core glycosyl unit as well as hyperglycosylation have rendered the preference of utilizing mammalian cells against yeast in generating therapeutic glycoproteins (Romanos, 1995; Stratton-Thomas *et al.*, 1995). Therefore a new antigen delivery system is crucial to avoid using carrier at the expense of antigen quality. Non-recombinant, *in-trans* binding of heterologous protein emerge to be an exciting solution for expression host restriction in surface display system. It can be hypothesized that therapeutic proteins such as TRP-2 can be produced in the mammalian cell system and then anchored to the bacterial *L. lactis* cell surface by fusing the cell wall anchoring motif, cA to the aforementioned therapeutic protein.
Therefore, the main objectives of this study is to express and purify a fusion protein comprising TRP-2 and C-terminal cell wall anchoring motif of *L. lactis* N-acetylmuramidase, cA in Chinese Hamster Ovary (CHO) cell system as well as to analyse its anchoring to live *L. lactis* cell wall.

The specific objectives are:

1. To construct vectors for the expression of *trp-2-cA* fusion gene and *trp-2* genes in mammalian CHO expression system.

2. To express the *trp*-2-*cA* and *trp*-2 genes in CHO cells and purify target TRP-2-cA protein;

3. To anchor purified TRP-cA fusion protein on the *L. lactis* cell wall.

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REFERENCES

Aebi, M., Bernasconi, R., Clerc, S. and Molinari, M. 2010. N-glycan structures: recognition and processing in the ER. *Trends Biochem Sci.*; 35(2):74-82.

al-Rubeai, M. and Singh, R. P. 1999. Apoptosis in cell culture. *Curr Opin Biotechnol* 9, 152-156.

Andre, G., Leenhouts, K., Hols, P. and Dufrene, Y. F. 2008. Detection and localization of single LysM-peptidoglycan interactions. *Journal of Bacteriology*. (190) 21: 7079-7086.

Anichini, A., Maccalli, C., Mortarini, R., Salvi, S., Mazzocchi, A., Squarcina, P., Herlyn, M. and Parmiani, G. 1993. Melanoma cells and normal melanocytes share antigens recognized by HLA- A2-restricted cytotoxic T cell clones from melanoma patients. *J Exp Med*; 177: 989–98.

Antikainen, J., Anton, L., Sillanpaa, J. and Korhönen, T. K. 2002. Domains in the S-layer protein CbsA of *Lactobacillus crispatus* involved in adherence to collagens, laminin and lipoteichoic acids and in self-assembly. *Molecular Microbiology* 46, 381–394.

Arhin, G.K., Boots, M., Bagga, P.S., Milcarek, C. and Wilusz, J. 2002. Downstream sequence elements with different affinities for the hnRNP H/H' protein influence the processing efficiency of mammalian polyadenylation signals. *Nucleic Acids Res.*; 30(8):1842-50.

Armstrong, J., Baddiley, J., Buchanan, J. G., and Carss, B. 1958. Nucleotides and the bacterial cell wall. *Nature* 181:1692–1693.

Aroca, P., Garcia-Borron, J.C., Solano, F. and Lozano, J.A. 1990. Regulation of mammalian melanogenesis. I: Partial purification and characterization of a dopachrome converting factor: dopachrome tautomerase. *Biochim Biophys Acta.*; 1035(3):266-75.

Arrighi, J.F., Barre, A., Ben Amor, B., Bersoult, A., Soriano, L.C., Mirabella, R., de Carvalho-Niebel, F., Journet, E.P., Ghérardi, M., Huguet, T., Geurts, R., Dénarié, J., Rougé, P. and Gough, C.. 2006. The *Medicago truncatula* lysin motif-receptor-like kinase gene family includes NFP and new nodule-expressed genes. *Plant Physiology* 142: 265–279.

Åvall-Jääskelainen, S. and Palva, A. 2006. Secretion of biologically active porcine interleukin-2 by *Lactococcus lactis*. *Vet. Microbiol*. (115) 1-3: 278-283.

Avogadri, F., Merghoub, T., Maughan, M.F., Hirschhorn-Cymerman, D., Morris, J., et.al. Ritter, E., Olmsted, R., Houghton, A.N. and Wolchok, J.D. 2010.

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Alphavirus Replicon Particles Expressing TRP-2 Provide Potent Therapeutic Effect on Melanoma through Activation of Humoral and Cellular Immunity. *PLoS ONE* 5(9): e12670.doi:10.1371/journal.pone.0012670

Baddiley, J. 1972. Teichoic acids in cell walls and membranes of bacteria. *Essays of Biochemistry*. 8:35–77.

Bahey-El-Din, M., G.M. Gahan, C., and T. Griffin, B. 2010. *Lactococcus lactis* as a Cell Factory for Delivery of Therapeutic Proteins. *Current Gene Therapy*, 10, 34-4.

Banz, A., Cremel, M., Mouvant, A., Guerin, N., Horand, F. and Godfrin, Y. 2012. Tumor growth control using red blood cells as the antigen delivery system and poly(I:C). *J Immunother*.;35(5):409-17.

Basrur, V., Yang, F., Kushimoto, T., Higashimoto, Y., Yasumoto, K., Valencia, J., Muller, J., Vieira, W.D., Watabe, H., Shabanowitz, J., Hearing, V.J., Hunt, D.F. and Appella, E. 2003. Proteomic analysis of early melanosomes: identification of novel melanosomal proteins. *J Proteome Res*. ; 2(1):69-79.

Bateman, A., and Bycroft, M. 2000. The structure of a LysM domain from *E. coli* membrane-bound lytic murein transglycosylase D (MltD). *Journal of Molecular Biology* 299: 1113–1119.

Benhar I. 2001. Biotechnological applications of phage and cell display. *Biotechnology Advances* 19(1):1-33.

Berg, J.M., Tymodczko, J.L. and Stryer, L. 2002. Biochemistry. New York: W. H. Freeman and Company. ISBN-10: 0-7167-3051-0.

Berger, T.G., Haendle, I., Schrama, D., Lüftl, M., Bauer, N., Pedersen, L.Ø., Schuler-Thurner, B., Hohenberger, W., Straten, Pt. Pt., Schuler, G. and Becker, J.C. 2004. Circulation and homing of melanoma-reactive T cells to both cutaneous and visceral metastases after vaccination with monocyte-derived dendritic cells. *Int J Cancer* ; 111: 229–37.

Bhavsar, A.P., Erdman, L.K., Schertzer, J.W. and Brown, E.D. 2004. Teichoic acid is an essential polymer in *Bacillus subtilis* that is functionally distinct from teichuronic acid. *Journal of Bacteriology*. 186:7865–7873.

Björkroth, J., and Koort, J. 2011. Lactic Acid Bacteria: Taxonomy and Biodiversity. Elsevier Ltd.

Boder, E.T. and Wittrup, K.D. 1997. Yeast surface display for screening combinatorial polypeptide libraries. *Nature Biotechnology*. 15 (1997) 553–557.

© COPYRIGHT UPM

Borovanský, J., and Riley, P.A. 2011. *Melanins and Melanosomes: Biosynthesis, Biogenesis, Physiological, and Pathological Functions*. Copyright © 2011 Wiley-VCH Verlag GmbH & Co. KGaAOnline ISBN: 9783527636150

Bosma, T., Kanninga, R., Neef, J., Audouy, S. A., van Roosmalen, M. L., Steen, A., Buist, G., Kok, J., Kuipers, O. P., Robillard, G. and Leenhouts, K. 2006. Novel surface display system for proteins on non-genetically modified gram-positive bacteria. *Appl. Environ. Microbiol*. (72) 1: 880-889.

Bradford M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72:248-54.

Braun, L., Dramsi, S., Dehoux, P., Bierne, H., Lindahl, G. and Cossart, P. 1997. InlB: an invasion protein of *Listeria monocytogenes* with a novel type of surface association. *Mol Microbiol* 25:285–294.

Brinkman, M., Walter, J., Grein, S., Thies, M.J., Schulz, T.W., Herrmann, M., Reiser, C.O. and Hess, J. 2005. Beneficial therapeutic effects with different particulate structures of murine polyomavirus VP1-coat protein carrying self or non-self CD8 T cell epitopes against murine melanoma. *Cancer Immunol Immunother*.; 54(6):611-22.

Bronte, V., Apolloni, E., Ronca, R., Zamboni, P., Overwijk, W.W., Surman, D.R., Restifo, N.P. and Zanovello, P. 2000. Genetic vaccination with "self" tyrosinase-related protein 2 causes melanoma eradication but not vitiligo. *Cancer Res.*; 60(2):253-8.

Bruhn, K.W., Craft, N., Nguyen, B.D., Yip, J. and Miller, J.F. 2005. Characterization of anti-self CD8 T-cell responses stimulated by recombinant *Listeria monocytogenes* expressing the melanoma antigen TRP-2. *Vaccine*.; 23(33):4263-72.

Buist, G., Karsens, H., Nauta, A., van Sinderen, D., Venema, G. and Kok , J. 1997. Autolysis of *Lactococcus lactis* caused by induced overproduction of its major autolysin, AcmA. *Applied Environmental Microbiology* 63, 2722–2728.

Buist, G., Kok, J., Leenhouts, K.J., Dabrowska, M., Venema, G., and Haandrikman, A.J. 1995. Molecular cloning and nucleotide sequence of the gene encoding the major peptidoglycan hydrolase of *Lactococcus lactis*, a muramidase needed for cell separation. *Journal of Bacteriology* 177: 1554–1563.

Buist, G., Steen, A., Kok, J. and Kuipers, O.P. 2008. LysM, a widely distributed protein motif for binding to (peptido)glycans. *Molecular Microbiology* ;68(4):838-47.

© COPYRIGHT UPM

Burda, P. and Aebi, M. 1999. The dolichol pathway of N-linked glycosylation. *Biochim Biophys Acta*, 1426:239-257.

Cabanes, D., Dussurget, O., Dehoux, P. and Cossart, P. 2004. Auto, a surface associated autolysin of *Listeria monocytogenes* required for entry into eukaryotic cells and virulence. *Molecular Microbiology* 51:1601–1614.

Cao, H.P., Wang, H.N., Yang, X., Zhang, A.Y., Li, X., Ding, M.D., Liu, S.T., Zhang, Z.K., and Yang F. 2013. *Lactococcus lactis* anchoring avian infectious bronchitis virus multi-epitope peptide EpiC induced specific immune responses in chickens. *Biosci Biotechnol Biochem*.; 77(7):1499-504.

Carroll, S.A., Hain, T., Technow, U., Darji, A., Pashalidis, P., Joseph, S.W. and Chakraborty, T. 2003. Identification and characterization of a peptidoglycan hydrolase, MurA, of *Listeria monocytogenes*, a muramidase needed for cell separation. *Journal of Bacteriology* 185:6801–6808.

Cassady, J.L. and Sturm, R.A. 1994. Sequence of the human dopachrome tautomerase-encoding TRP-2 cDNA. *Gene*. ; 143(2):295-8.

Charbit, A., Boulain, J.C., Ryter, A. and Hofnung, M., 1986. Probing the topology of a bacterial membrane protein by genetic insertion of a foreign epitope; expression at the cell surface. *EMBO J*. 5, 3029–3037.

Chu, W., Pak, B.J., Bani, M.R., Kapoor, M., Lu, S.J., Tamir, A., Kerbel, R.S., Ben-David.,Y. 2000. Tyrosinase-related protein 2 as a mediator of melanoma specific resistance to cis-diamminedichloroplatinum(II): therapeutic implications. *Oncogene* 19:395–402.

Chauvaux, S., Matuschek, M. and Beguin, P. 1999. Distinct affinity of binding sites for S-layer homologous domains in *Clostridium thermocellum* and *Bacillus anthracis* cell envelopes. *Journal of Bacteriology* 181: 2455–2458.

Chi, A., Valencia, J.C., Hu, Z.Z., Watabe, H., Yamaguchi, H., Mangini, N.J., Huang, H., Canfield, V.A., Cheng, K.C., Yang, F., Abe, R., Yamagishi, S., Shabanowitz, J., Hearing, V.J., Wu, C., Appella, E. and Hunt, D.F. Proteomic and bioinformatic characterization of the biogenesis and function of melanosomes. *J Proteome Res.*; 5(11):3135-44.

Cloosen, S., Arnold, J., Thio, M., Bos, G.M., Kyewski, B. and Germeraad, W.T. 2007. Expression of tumor-associated differentiation antigens, MUC1 glycoforms and CEA,in human thymic epithelial cells: implications for self-tolerance and tumor therapy. *Cancer Res*; 67(8):3919–26.

Cortes-Perez, N.G., Azevedo, V., Alcocer-González, J.M., Rodriguez-Padilla, C., Tamez-Guerra, R.S, Corthier, G., Gruss, A., Langella, P. and Bermúdez-

© COPYRIGHT UPM

Humarán, L.G. 2005. Cell-surface display of E7 antigen from human papillomavirus type-16 in *Lactococcus lactis* and in *Lactobacillus plantarum* using a new cell-wall anchor from lactobacilli. *J Drug Target*.;13(2):89-98.

Costin, G.E., Valencia, J.C., Wakamatsu, K., Ito, S., Solano, F., Milac, A.L., Vieira, W.D., Yamaguchi, Y., Rouzaud, F., Petrescu, A.J., Lamoreux, M.L. and Hearing, V.J. 2005. Mutations in dopachrome tautomerase (Dct) affect eumelanin/pheomelanin synthesis, but do not affect intracellular trafficking of the mutant protein. *Biochem J* 391:249–259

Darzacq, X., Singer R.H. and Shav-Tal, Y. 2005. Dynamics of transcription and mRNA export. *Curr Opin Cell Biol*.; 17(3):332-9.

Davila, E., Kennedy, R. and Celis, E. 2003. Generation of antitumor immunity by cytotoxic T lymphocyte epitope peptide vaccination, CpG-oligodeoxynucleotide adjuvant, and CTLA-4 blockade. *Cancer Res.*; 63(12):3281-8.

DePalma, B. 2013. Twisting and Turning for Better Protein Expression. GeneticEngineering & Biotechnology News. Featured Articles (Vol. 33, No. 1) http://www.genengnews.com/gen-articles/twisting-and-turning-for-better-protein-expression/4666/ (accessed 2nd August 2014).

Dermime, S., Gilham, DE., Shaw, D.M., Davidson, E.J., Meziane el, K., Armstrong, A., Hawkins, R.E. and Stern P.L. 2004.Vaccine and antibody-directed T cell tumour immunotherapy. *Biochim Biophys Acta*;1704(1):11–35.

Derouazi, M., Wang, Y., Marlu, R., Epaulard, O., Mayol, J.F., Pasqual, N., Le Gouellec, A., Polack, B. and Toussaint, B. 2010. Optimal epitope composition after antigen screening using a live bacterial delivery vector: application to TRP-2. *Bioeng Bugs*. 2010;1(1):51-60.

Desvaux, M., Dumas, E., Chafsey, I. and Hébraud, M. 2006. Protein cell surface display in Gram-positive bacteria: from single protein to macromolecular protein structure. *FEMS Microbiology Letters* ;256(1):1-15.

Eggert, A.A., Schreurs, M.W., Boerman, O.C., Oyen, W.J., de Boer, A.J, Punt, C.J., Figdor, C.G. and Adema, G.J. 1999. Biodistribution and vaccine efficiency of murine dendritic cells are dependent on the route of administration. *Cancer Res*.; 59(14):3340-5.

Eggert, A.O., Becker, J.C., Ammon, M., McLellan, A.D., Renner, G., Merkel, A., Bröcker, E.B. and Kämpgen, E. 2002. Specific peptide-mediated immunity

© COPYRIGHT UPM

against established melanoma tumors with dendritic cells requires IL-2 and fetal calf serum-free cell culture. *Eur J Immunol*.; 32(1):122-7.

Faiger, H. and Ivanchenko, M. and Haran, T.E. 2007. Nearest-neighbor non-additivity versus long-range non-additivity in TATA-box structure and its implications for TBP-binding mechanism. *Nucleic Acids Res.*; 35(13):4409-19.

Fischer, W. 1994. Lipoteichoic acid and lipids in the membrane of *Staphylococcus aureus*. *Medical Microbiology and Immunology* 183: 61–76.

Foster, S. 1991. Cloning, expression, sequence analysis and biochemical characterization of an autolytic amidase of *Bacillus subtilis* 168 trpC2. *J Gen Microbiol* 137:1987–1998.

Fouet A. 2009. The surface of *Bacillus anthracis*. *Molecular Aspects of Medicine*. 30:374–385.

Fox, P.F. 2011. Lactic Acid Bacteria: An Overview. Elsevier Ltd.

Freudl, R., MacIntyre, S., Degen, M., Henning, U., 1986. Cell surface exposure of the outer membrane protein OmpA of *Escherichia coli* K-12. *Journal of Molecular Biology*. 188, 491–494.

Fukui, M., Nakano-Hashimoto, T., Okano, K., Maruta, Y., Suehiro, Y., Hamanaka, Y., Yamashita, H., Imai, K., Kawano, M.M. and Hinoda, Y. 2004. Therapeutic effect of dendritic cells loaded with a fusion mRNA encoding tyrosinase-related protein 2 and enhanced green fluorescence protein on B16 melanoma. *Tumour Biol*.; 25(5-6):252-7.

Fukushima, S., Hirata, S., Motomura, Y., Fukuma, D., Matsunaga, Y., Ikuta, Y., Ikeda, T., Kageshita, T., Ihn, H., Nishimura, Y. and Senju, S. 2009. Multiple antigen-targeted immunotherapy with alpha-galactosylceramide-loaded and genetically engineered dendritic cells derived from embryonic stem cells. *J Immunother*.; 32(3):219-31.

Garcia, E., Garcia, J.L., Garcia, P., Arraras, A., Sanchez-Puelles, J.M. and Lopez, R. 1988. Molecular evolution of lytic enzymes of Streptococcus pneumoniae and its bacteriophages. *Proceeding of the National Academy of Science USA* 85:914–918.

Garcia, E., Garcia, J.L., Ronda, C., Garcia, P. and Lopez, R. 1985. Cloning and expression of the pneumococcal autolysin gene in *Escherichia coli*. *Mol Gen Genet* 201:225–230.

Garcia, P., Gonzalez, M.P., Garcia, E., Lopez, R. and Garcia, J.L. 1999a. LytB, a novel pneumococcal murein hydrolase essential for cell separation. *Molecular Microbiology* 31:1275–1281.

© COPYRIGHT UPM

Garcia, P., Paz Gonzalez, M., Garcia, E., Garcia, J.L. and Lopez, R. 1999b. The molecular characterization of the first autolytic lysozyme of *Streptococcus pneumoniae* reveals evolutionary mobile domains. *Molecular Microbiology* 33:128–138.

Garvey, K.J., Saedi, M.S., and Ito, J. 1986. Nucleotide sequence of Bacillus phage phi 29 genes 14 and 15: homology of gene 15 with other phage lysozymes. *Nucleic Acids Research* 14: 10001–10008.

Gasson, M.J. 1983. Plasmid complements of *Streptococcus lactis* NCDO 712 and other lactic streptococci after protoplast-induced curing. *J Bacteriol* ; 154(1): 1–9.

Georgiou, G., Stathopoulos, C., Daugherty, P.S., Nayak, A.R., Iverson, B.L. and Curtiss, R.I., 1997. Display of heterologous proteins on the surface of microorganisms: from the screening of combinatorial libraries to live recombinant vaccines. *Nature Biotechnology*. 15, 29–34.

Giarelli, E., 2007. Cancer vaccines: a new frontier in prevention and treatment, *Oncology (Williston Park)* 21 11. Oct; 21(11 Suppl Nurse Ed):11-7; discussion 18.

Giffard, P.M. and Jacques, N.A. 1994. Definition of a fundamental repeating unit in streptococcal glucosyltransferase glucan-binding regions and related sequences. *Journal of Dental Research.*; 73(6):1133-41.

Gilboa, E. 2001. The risk of autoimmunity associated with tumor immunotherapy. *Nat Immunol* 2(9): 789–92.

Glover, D.J., Lipps, H.J. and Jans, D.A. 2005. Towards safe, non-viral therapeutic gene expression in humans. *Nat Rev Genet* 6(4): 299–310.

Gregor, P.D., Wolchok, J.D., Ferrone, C.R., Buchinshky, H., Guevara-Patiño, J.A, Perales, M.A, Mortazavi, F., Bacich, D., Heston, W., Latouche, J.B, Sadelain, M., Allison, J.P., Scher H.I. and Houghton A.N. 2004. CTLA-4 blockade in combination with xenogeneic DNA vaccines enhances T-cell responses, tumor immunity and autoimmunity to self antigens in animal and cellular model systems. *Vaccine*.; 22(13-14):1700-8.

Grinshtein, N., Ventresca, M., Margl, R., Bernard, D., Yang, T.C., Millar, J.B., Hummel, J., Beermann, F., Wan, Y. and Bramson, J.L. 2009. High-dose chemotherapy augments the efficacy of recombinant adenovirus vaccines and improves the therapeutic outcome. *Cancer Gene Ther*.; 16(4):338-50.

© COPYRIGHT UPM

Guimaraes, V. D., Gabriel, J. E., Lefevre, F., Cabanes, D., Gruss, A., Cossart, P., Azevedo, V. and Langella, P. 2005. Internalin-expressing *Lactococcus lactis* is able to invade small intestine of guinea pigs and deliver DNA into mammalian epithelial cells. *Microbes Infect*. (7) 5-6: 836-844.

Guiral, S., Mitchell, T.J., Martin, B and Claverys, J.P. 2005. Competence-programmed predation of noncompetent cells in the human pathogen *Streptococcus pneumoniae*: genetic requirements. *Proceeding of the National Academy of Science USA* 102: 8710–8715.

Hamdy, S., Haddadi, A., Hung, R.W. and Lavasanifar, A. 2011. Targeting dendritic cells with nano-particulate PLGA cancer vaccine formulation. *Advanced Drug Delivery Reviews.* ; 63(10-11):943-55.

Hamdy, S., Molavi, O., Ma, Z., Haddadi, A., Alshamsan, A., Gobti, Z., Elhasi, S. and Samuel, J., Lavasanifar, A. 2008. Co-delivery of cancer-associated antigen and Toll-like receptor 4 ligand in PLGA nanoparticles induces potent CD8+ T cell-mediated anti-tumor immunity. *Vaccine*.; 26(39):5046-57.

Hawkins, W.G., Gold, J.S., Blachere, N.E., Bowne, W.B., Hoos, A., Lewis, J.J. and Houghton, A.N. 2002. Xenogeneic DNA immunization in melanoma models for minimal residual disease. *J Surg Res*.; 102(2):137-43.

Helenius, A. and Aebi, M. 2004. Roles of N-linked glycans in the endoplasmic reticulum. *Annu Rev Biochem*.; 73:1019-49.

Hesketh, J.E. and Pryme, I.F. 1991. Interaction between mRNA, ribosomes and the cytoskeleton. *Biochem J*. ; 277 (Pt 1):1-10.

Horsburgh, G.J., Atrih, A., Williamson, M.P. and Foster, S.J. 2003. LytG of *Bacillus subtilis* is a novel peptidoglycan hydrolase: the major active glucosaminidase. *Biochemistry* 42:257–264.

Hu, S., Li, L., Qiao, J., Guo, Y. Cheng, L. and Liu, J. 2006. Codon optimization, expression, and characterization of an internalizing anti-ErbB2 single-chain antibody in *Pichia pastoris*. *Protein Expr Purif*.; 47(1):249-57.

Huard, C., Miranda, G., Redko, Y., Wessner, F., Foster, S.J. and Chapot-Chartier, M.P. 2004. Analysis of the peptidoglycan hydrolase complement of *Lactococcus lactis*: identification of a third N-Acetylglucosaminidase, AcmC. *Applied Environmental Microbiology* 70:3493–3499.

Huard, C., Miranda, G., Wessner, F., Bolotin, A., Hansen, J., Foster, S.J. and Chapot-Chartier, M-P. 2003. Characterization of AcmB, an N-acetylglucosaminidase autolysin from *Lactococcus lactis*. *Microbiology* 149:695–705.

© COPYRIGHT UPM

Huber, C., Ilk, N., Runzler, D., Egelseer, E.M., Weigert, S., Sleytr, U.B. and Sara, M. 2005. The three S-layer-like homology motifs of the S-layer protein SbpA of *Bacillus sphaericus* CCM 2177 are not sufficient for binding to the pyruvylated secondary cell wall polymer. *Molecular Microbioly* 55: 197–205.

Imperiali, B., Shannon, K.L., and Rickert, K.W. 1992. Role of peptide conformation in asparagine-linked glycosylation. *J. Am. Chem. Soc.*, 114, 7042–7044.

Innocentin, S., Guimaraes, V., Miyoshi, A., Azevedo, V., Langella, P., Chatel, J. M. and Lefevre, F. 2009. *Lactococcus lactis* expressing either *Staphylococcus aureus* fibronectin-binding protein A or *Listeria monocytogenes* internalin A can efficiently internalize and deliver DNA in human epithelial cells. *Appl. Environ. Microbio*l. (75) 14: 4870-4878.

InSug, O., Blaszczyk-Thurin, M., T.Shen, C. and CJ Ertl, H. 2003. A DNA vaccine expressing tyrosinase-related protein 2 induces T-cell-mediated protection against mouse glioblastoma. *Cancer Gene Therapy* 10, 678-688.

Ishikawa, S., Yamane, K. and Sekiguchi, J. 1998. Regulation and characterization of a newly deduced cell wall hydrolase gene (cwlJ) which affects germination of *Bacillus subtilis* spores. *J Bacteriol* 180:1375–1380.

Jayapal, K.P., Wlaschin, K.F., Hu, W-S. and Yapm M.G.S. 2007. Recombinant protein therapeutics from Cho Cells—20 years and counting. *CHO Consortium: SBE Special Edition*: 40–47.

Jia, Z.C., Zou, L.Y., Ni, B., Wan, Y., Zhou, W., Lv, Y.B, Geng, M., Wu, Y.Z. 2005. Effective induction of antitumor immunity by immunization with plasmid DNA encoding TRP-2 plus neutralization of TGF-beta. *Cancer Immunology and Immunotherapy*.;54(5):446-52.

Johnson, A.E. and van Waes, M.A. 1999. The translocon: a dynamic gateway at the ER membrane. *Annu Rev Cell Dev Biol*.; 15:799-842.

Kajikawa, A., Masuda, K.,, Mitsunori, K., and Shizunobu, I. 2010. Adjuvant Effects for Oral Immunization Provided by Recombinant *Lactobacillus casei* Secreting Biologically Active Murine Interleukin-1. *Clinical and Vaccine Immunology*, p. 43–48.

Kajimura, J., Fujiwara, T., Yamada, S., Suzawa, Y., Nishida, T., Oyamada, Y., Hayashi, I., Yamagishi, J-I., Komatsuzawa, H., Sugai, M. 2005. Identification and molecular characterization of an N-acetylmuramyl-L-alanine amidase Sle1 involved in cell separation of *Staphylococcus aureus*. *Molecular Microbiology* 58:1087–1101.

Kang, T.H., Mao, C.P., La, V., Chen, A., Hung, C.F. and Wu, T.C. 2013. Innovative DNA vaccine to break immune tolerance against tumor self-antigen. *Hum Gene Ther*.; 24(2):181-8.

© COPYRIGHT UPM

Kelkar, S. M., Shenoy, M. S. and Kaklij, G. S. 1988. Antitumour activity of lactic acid bacteria on a solid fibrosarcoma, sarcoma-180 and Ehrlich ascites carcinoma. *Cancer Lett*., 42, 73–77.

Kim, T.K. and Eberwine, J.H. 2010. Mammalian cell transfection: the present and the future. *Anal Bioanal Chem*. ;397(8):3173-8.

Kimura, T., Koya, R.C., Anselmi, L., Sternini, C., Wang, H.J, Comin-Anduix, B., Prins, R.M., Faure-Kumar, E., Rozengurt, N., Cui, Y., Kasahara, N., and Stripecke, R. 2007. Lentiviral vectors with CMV or MHCII promoters administered in vivo: immune reactivity versus persistence of expression. *Mol Ther*.; 15(7):1390-9.

König, H., Claus H. and Varma A. 2010. *Prokaryotic Cell Wall Compounds Structure and Biochemistry*. Springer Heidelberg Dordrecht London New York ISBN 978-3-642-05061-9.

Kuroda, A. and Sekiguchi, J. 1991. Molecular cloning and sequencing of a major *Bacillus subtilis* autolysin gene. *J Bacteriol* 173:7304–7312.

Kuroda, A., Asami, Y. and Sekiguchi, J. 1993. Molecular cloning of a sporulation-specific cell wall hydrolase gene of *Bacillus subtilis*. *J Bacteriol* 175:6260–6268.

Kuroda, A., Imazeki, M. and Sekiguchi, J. 1991. Purification and characterization of a cell wall hydrolase encoded by the cwlA gene of *Bacillus subtilis*. *FEMS Microbiol Lett* 65:9–13.

Kuroda, A., Sekiguchi, J. 1991. Molecular cloning and sequencing of a major *Bacillus subtilis* autolysin gene. *Journal of Bacteriology* ;173(22):7304-12.

Kwaks, T.H. and Otte, A.P. 2006. Employing epigenetics to augment the expression of therapeutic proteins in mammalian cells. *Trends Biotechnol*. (3):137-42.

Åvall-Jääskeläinen, K., Alakuijala, U. and Saris, P. E. 2010. Immobilization of *Lactococcus lactis* to cellulosic material by cellulose-binding domain of *Cellvibrio japonicus*. *J. Appl. Microbiol*. 109:1274–1283.

Lei, E.P. and Silver, P.A. 2002. Intron status and 3'-end formation control cotranscriptional export of mRNA. *Genes Dev*.; 16(21):2761-6.

Leoff, C., Choudhury, B., Saile, E., Quinn, C.P., Carlson, R.W. and Kannenberg, E.L. 2008. Structural Elucidation of the non-classical secondary cell wall polysaccharide from *Bacillus cereus* ATCC 10987: Comparison with the polysaccharides from *Bacillus anthracis* and *B. cereus* type strain ATCC 14579 reveals both unique and common structural features. *Journal of Biology Chemistry*. 283: 29812–29821.

© COPYRIGHT UPM

Lim, S.H., Jahanshiri, F., Rahim, R.A, Sekawi, Z. and Yusoff, K. 2010. Surface display of respiratory syncytial virus glycoproteins in *Lactococcus lactis* NZ9000. *Lett Appl Microbiol* ; 51(6):658-64.

Lin, J.J. 1992. Endonuclease A degrades chromosomal and plasmid DNA of *Escherichia coli* present in most preparations of single stranded DNA from phagemids. *Proc. Natl. Sci. Counc. Repub. China* B 16 1-5.

Lindholm A, Smeds A, and Palva A. 2004. Receptor binding domain of *Escherichia coli* F18 fi mbrial adhesin FedF can be both efficiently secreted and surface displayed in a functional form in *Lactococcus lactis. Appl. Environ. Microbiol.* 70(4): 2061-2071.

Liu, G., Akasaki, Y., T. Khong, H., J Wheeler, C., Das, A., L. Black, K., S. Yu, J. 2005. Cytotoxic T cell targeting of TRP-2 sensitizes human malignant glioma to chemotherapy. *Oncogen*e, 24, 5226-5234.

Low, L.Y., Yang, C., Perego, M., Osterman, A. and Liddington, R. 2011. Role of net charge on catalytic domain and influence of cell wall binding domain on bactericidal activity, specificity, and host range of phage lysins. *The Journal of Biology Chemistry*. ;286(39): 34391-403.

Lowe, J. B. and Marth, J. D. 2003. A genetic approach to mammalian glycan function. *Annu. Rev. Biochem*. 72, 643–691.

Lucas, S. and Coulie, P.G. 2008. About human tumor antigens to be used in immunotherapy. *Semin Immunol*.; 20(5):301-7.

Ludewig, B., Ochsenbein, A.F., Odermatt, B., Paulin, D., Hengartner, H., Zinkernagel, R.M. 2000. Immunotherapy with dendritic cells directed against tumor antigens shared with normal host cells results in severe autoimmune disease. *J Exp Med*; 191(5): 795–804.

Mahnke, K., Qian, Y. Fondel, S., Brueck, J., Becker, C. and Enk, A.H. 2005. Targeting of antigens to activated dendritic cells in vivo cures metastatic melanoma in mice. *Cancer Res*.; 65(15): 7007-12.

Marinus, M.G. 1996. *Escherichia coli* and *Salmonella*: Cellular and Molecular Biology, Second Edition, Neidhardt, F.C., ed., ASM Press, Washington, D.C.

Margot, P. and Karamata, D. 1992. Identification of the structural genes for N-acetylmuramoyl-L-alanine amidase and its modifier in *Bacillus subtilis* 168: inactivation of these genes by insertional mutagenesis has no effect on growth or cell separation. *Mol Gen Genet* 232:359–366.

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Margot, P., Pagni, M., Karamata, D. 1999. *Bacillus subtilis* 168 gene lytF encodes a gamma-D-glutamate-meso-diaminopimelate muropeptidase expressed by the alternative vegetative sigma factor, sigmaD. *Microbiology* 145:57–65.

Margot, P., Wahlen, M., Gholamhoseinian, A., Piggot, P., Karamata, D. and Gholamhuseinian, A. 1998. The lytE gene of *Bacillus subtilis* 168 encodes a cell wall hydrolase. *J Bacteriol* 180:749–75.

Mattner, F., Fleitmann, J.K., Lingnau, K., Schmidt, W., Egyed, A., Fritz, J., Zauner, W., Wittmann, B., Gorny, I., Berger, M., Kirlappos, H., Otava, A., Birnstiel, M.L. and Buschle, M. 2002. Vaccination with poly-L-arginine as immunostimulant for peptide vaccines: induction of potent and long-lasting T-cell responses against cancer antigens. *Cancer Res*.; 62(5):1477-80

.

McCormick, A.A., Corbo, T.A., Wykoff-Clary, S., Palmer, K.E. and Pogue, G.P. 2006. Chemical conjugate TMV-peptide bivalent fusion vaccines improve cellular immunity and tumor protection. *Bioconjug Chem*.; 17(5):1330-8.

Mego, M., Ebringer, L., Drgona, L., Mardiak, J., Trupl, J., Greksak, R., Nemova, I., Oravcova, E., Zajac, V., Koza, I. 2005. Prevention of febrile neutropenia in cancer patients by probiotic strain *Enterococcus faecium* M-74. *Pilot study phase I Neoplasma, 52 (2005),* pp. 159–164.

Mellquist, J.L., Kasturi, L., Spitalnik, S.L. and Shakin-Eshleman, S.H. 1998. The amino acid following an asn-X-Ser/Thr sequon is an important determinant of N-linked core glycosylation efficiency. *Biochemistry*.; 37(19):6833-7.

Mendiratta, S.K., Thai, G., Eslahi, N.K., Thull, N.M., Matar, M., Bronte, V. and Pericle, F. 2001. Therapeutic tumor immunity induced by polyimmunization with melanoma antigens gp100 and TRP-2. *Cancer Res*.; 61(3):859-63.

Mercenier, A., Müller-Alouf, H., Grangette, C. 2000. Lactic acid bacteria as live vaccines. *Current Issues in Molecular Biology* 2:17-25.

Meroueh, S. O., Bencze, K. Z., Hesek, D., Lee, M., Fisher, J. F., Stemmler, T. L., and Mobashery, S. 2006. Three-dimensional structure of the bacterial cell wall peptidoglycan. *Proceedings of the National Academy of Science. U.S.A*. 103, 4404–4409.

Michard, Q., Commo, S., Belaidi, J.P., Alleaume, A.M., Michelet, J.F., Daronnat, E., Eilstein, J., Duche, D., Marrot, L., and Bernard, B.A. 2008. TRP-2 specifically decreases WM35 cell sensitivity to oxidative stress. *Free Radic Biol Med*.;44(6):1023-31.

© COPYRIGHT UPM

Miconnet, I., Coste, I., Beermann, F., Haeuw, J.F., Cerottini, J.C., Bonnefoy, J.Y., Romero, P. and Renno, T. 2001. Cancer vaccine design: a novel bacterial adjuvant for peptide-specific CTL induction. *J Immunol*.; 166(7): 4612-9.

Mills, S. and Ross, R.P. 2011. *Lactococcus lactis*. Elsevier Ltd.

Moeini, H., Rahim, R.A., Omar, A.R., Shafee, N. and Yusoff, K. 2011. *Lactobacillus acidophilus* as a live vehicle for oral immunization against chicken anemia virus. *Appl Microbiol Biotechnol* 90:77–88.

Molinari, M. 2007 . N-glycan structure dictates extension of protein folding or onset of disposal. *Nat. Chem. Bi*ol. 3: 313 – 320 .

Morello, E., Bermudez-Humaran, L.G., Llull, D., Sole, V., Miraglio, N., Langella, P. and Poquet, I. 2008. *Lactococcus lactis*, an Efficient Cell Factory for Recombination Protein Production and Secretion. J*ournal of Molecular Microbiology and Biotechnology* 14:48-58.

Moriyama, R., Hattori, A., Miyata, S., Kudoh, S. and Makino, S. 1996. A gene (sleB) encoding a spore cortex-lytic enzyme from *Bacillus subtilis* and response of the enzyme to L-alanine-mediated germination. *J Bacteriol* 178:6059–6063.

Mozzi, F., Raya, R.R. and Vignolo G.M. 2010. *Biotechnology of Lactic Acid Bacteria Novel Applications*. Wiley-Blackwell. ISBN: 978-0-8138-1583-1.

Murshid, A. and Presley, J.F. 2004. ER-to-Golgi transport and cytoskeletal interactions in animal cells. *Cell Mol Life Sci.*; 61(2):133-45.

Narum, D.L., Kumar, S., Rogers, W.O., Fuhrmann, S.R., Liang, H., Oakley, M., Taye, A., Sim, B.K. and Hoffman, S.L. 2001. Codon optimization of gene fragments encoding *Plasmodium falciparum* merzoite proteins enhances DNA vaccine protein expression and immunogenicity in mice. *Infect Immun.*; 69(12):7250-3.

Nasser, M.W. 2003. Evaluation of Yeast as an Expression System. *Indian Journal of Biotechnology*. Vol 2, pp 477-493.

Navarre, W.W. and Schneewind, O. 1999. Surface proteins of gram-positive bacteria and mechanisms of their targeting to the cell wall envelope. *Microbiology and Molecular Biology Review*. 1999 Mar;63(1):174-229.

Neeson, P. and Paterson, Y. 2006. Effects of the tumor microenvironment on the efficacy of tumor immunotherapy, *Immunol. Invest*. 35 359.

Negriou, G., Dwek, D.A. and Petrescu, S.M. 2003. The inhibition of early N-glycan processing targets TRP-2 to degradation in B16 melanoma cells. *The Journal Biological Chemistry* 278(29):27035-42.

© COPYRIGHT UPM

Ng, W.L., Kazmierczak, K.M., Winkler, M.E. 2004. Defective cell wall synthesis in *Streptococcus pneumoniae* R6 depleted for the essential PcsB putative murein hydrolase or the VicR (YycF) response regulator. *Mol Microbiol* 53:1161–1175.

Niethammer A.G, Xiang, R., Ruehlmann, J.M., Lode, H.N., Dolman, C.S., Gillies, S.D. and Reisfeld, R.A. 2001. Targeted interleukin 2 therapy enhances protective immunity induced by an autologous oral DNA vaccine against murine melanoma. *Cancer Res*.; 61(16):6178-84.

Norton, P. M., Brown, H. W., Wells, J. M., Macpherson, A. M., Wilson, P. W. and Le Page, R. W. 1996. Factors affecting the immunogenicity of tetanus toxin fragment C expressed in *Lactococcus lactis*. *FEMS Immunol. Med. Microbiol*. (14) 2-3: 167-177.

Nugroho, F.A., Yamamoto, H., Kobayashi, Y. and Sekiguchi, J. 1999. Characterization of a new sigma-Kdependent peptidoglycan hydrolase gene that plays a role in Bacillus subtilis mother cell lysis. *J Bacteriol* 181:6230–6237.

Ohnuma, T., Onaga, S., Murata, K., Taira, T., and Katoh, E. 2008. LysM domains from *Pteris ryukyuensis* chitinase-A: a stability study and characterization of the chitin-binding site. *Journal of Biology Chemistry* 283: 5178–5187.

Okamoto, T., Irie, R.F., Fujii, S., Huang, S.K., Nizze, A.J., Morton, D.L. and Hoon, D.S. 1999. Anti-tyrosinase-related protein-2 immune response in vitiligo patients and melanoma patients receiving active-specific immunotherapy. *J Invest Dermatol*.; 111(6):1034-9.

Orphanides, G. and Reinberg, D., 2002. A unified theory of gene expression. *Cell*.; 108(4):439-51.

Oshida T., Sugai M., Komatsuzawa H., Hong Y.M., Suginaka H. and Tomasz A. 1995. A *Staphylococcus aureus* autolysin that has an N-acetylmuramoyl-L-alanine amidase domain and an endo-beta- N-acetylglucosaminidase domain: cloning, sequence analysis, and characterization. *Proceeding of the National Academy of Science USA* 92:285–289.

Ostankovitch, M., Altrich-Vanlith, M., Robila, V. and Engelhard, V.H. 2009. N-glycosylation enhances presentation of a MHC class I-restricted epitope from tyrosinase. *Journal of Immunology* 182:4830–4835

Pagliero, E., Dideberg, O., Vernet, T. and Di Guilmi, A.M. 2005. The PECACE domain: a new family of enzymes with potential peptidoglycan cleavage activity in Gram-positive bacteria. *BMC Genomics* 6:19

© COPYRIGHT UPM

Pak, B.J., Lee, J., Thai, B.L., Fuchs, S.Y., Shaked, Y., Ronai, Z., Kerbel, R.S. and Ben-David, Y. 2004. Radiation resistance of human melanoma analysed by retroviral insertional mutagenesis reveals a possible role for dopachrome tautomerase. *Oncogene*.; 23(1):30-8.

Parkhurst, M.R., DePan, C., Riley, J.P., Rosenberg, S.A. and Shu, S. 2003. Hybrids of dendritic cells and tumor cells generated by electrofusion simultaneously present immunodominant epitopes from multiple human tumor-associated antigens in the context of MHC class I and class II molecules. *J Immunol*.; 170(10):5317-25.

Pejawar-Gaddy S. and J. Finn, O. 2008. Cancer vaccines: Accomplisments and Challenges. *Critical Reviews in Oncology Hematology* 93-102.

Piard, J. C., Hautefort, I., Fischetti, V. A., Ehrlich, S. D., Fons, M. and Gruss, A. 1997. Cell wall anchoring of the *Streptococcus pyogenes* M6 protein in various lactic acid bacteria. *J. Bacteriol.* (179) 9: 3068-3072.

Plotkin, J.B. and Kudla, G. 2011. Synonymous but not the same: the causes and consequences of codon bias. *Nat Rev Genet*.; 12(1):32-42.

Ponnambalam, S. and Baldwin, S.A. 2003. Constitutive protein secretion from the trans-Golgi network to the plasma membrane. *Mol Membr Biol*.; 20(2):129-39.

Pontes, D.S., de Azevedo, M.S.P., Chatel, J-M., Langella, P., Azevedo, V. and Miyoshi, A. 2011. *Lactococcus lactis* as live vector: Heterologous protein production and DNA delivery systems. *Protein Expression and Purification*; 79(2):165-75.

Ponting, C.P., Aravind, L., Schultz, J., Bork, P., and Koonin, E.V. 1999. Eukaryotic signalling domain homologues in archaea and bacteria. Ancient ancestry and horizontal gene transfer. *Journal of Molecular Biology* 289: 729–745.

Poquet, I., Saint, V., Seznec, E., Simoes, N., Bolotin, A., Gruss, A. 2000. HtrA is the unique surface housekeeping protease in *Lactococcus lactis* and is required for natural protein processing. *Molecular Microbiology* 35:1042–1051.

Pozzi, G., Contorni, M., Oggioni, M.R., Manganelli, R., Tommasino, M., Cavalieri, F., Fischetti, V.A. 1992. Delivery and expression of a heterologous antigen on the surface of streptococci. *Infect Immun* ; 60(5):1902-7.

Proudfoot, N.J., Furger, A. and Dye, M.J. 2002. Integrating mRNA processing with transcription. *Cell*.; 108(4): 501-12.

Pryme, I.F., Partridge, K., Johannessen, A.J., Jodar, D., Tauler, A. and Hesketh, J.E. 1996. Compartmentation of the protein synthetic machinery of CHO cells into

© COPYRIGHT UPM

free, cytoskeletal-bound and membrane-bound polysomes. *Gen Eng Biotechnol*.; 16:137–144.

Que, Y. A., Francois, P., Haefl iger, J. A., Entenza, J. M., Vaudaux, P. and Moreillon, P. 2001. Reassessing the role of *Staphylococcus aureus* clumping factor and fibronectin-binding protein by expression in *Lactococcus lactis*. *Infect. Immun*. (69) 10: 6296-6302.

Que, Y. A., Haefl iger, J. A., Francioli, P. and Moreillon, P. 2000. Expression of *Staphylococcus aureus* clumping factor A in *Lactococcus lactis* subsp. cremoris using a new shuttle vector. *Infect. Immun* (68) 6: 3516-3522.

Que, Y. A., Haefl iger, J. A., Piroth, L., Francois, P., Widmer, E., Entenza, J. M., Sinha, B., Herrmann, M., Francioli, P., Vaudaux, P. and Moreillon, P. 2005. Fibrinogen and fibronectin binding cooperate for valve infection and invasion in *Staphylococcus aureus* experimental endocarditis. *J. Exp. Med.* (201) 10: 1627-1635.

Rad H.H., Yamashita T., Jin H-Y., Hirosaki K., Wakamatsu K., Ito S. and Jimbow, K. 2004. Tyrosinase-related proteins suppress tyrosinase mediated cell death of melanocytes and melanoma cells. *Experimental Cell Research* 298; 317 – 328.

Rademaker, J.L, Herbet, H., Starrenburg, M.J, Naser, S.M., Gevers, D., Kelly, W.J., Hugenholtz, J., Swings, J., van Hylckama Vlieg, J.E. 2007. Diversity analysis of dairy and nondairy *Lactococcus lactis* isolates, using a novel multilocus sequence analysis scheme and (GTG)5-PCR fingerprinting. *Applied and Environmental Microbiology* 73: 7128–7137.

Radutoiu, S., Madsen, L.H., Madsen, E.B., Jurkiewicz, A., Fukai, E., Quistgaard, E.M., Albrektsen, A.S., James, E.K., Thirup, S., Stougaard, J. 2007. LysM domains mediate lipochitin-oligosaccharide recognition and Nfr genes extend the symbiotic host range. *The EMBO Journal* 26: 3923–3935.

Raha A.R., Varma N.R.S., Yusoff K., Ross E. and Foo H.L. 2005. Cell surface display system for *Lactococcus lactis*: a novel development for oral vaccine. *Appl Microbiol Biotechnol* 68:75–81.

Raleigh, E.A. and Wilson, G., 1986. *Escherichia coli* K-12 restricts DNA containing 5-methylcytosine. *Proc. Natl. Acad. Sci. USA* 83, 9070–4.

Ramadurai, L. and Jayaswal, R.K. 1997. Molecular cloning, sequencing, and expression of lytM, a unique autolytic gene of *Staphylococcus aureus*. *Journal of Bacteriology* 179:3625–3631.

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

© COPYRIGHT UPM

Recillas-Targa, F. 2006. Multiple strategies for gene transfer, expression, knockdown, and chromatin influence in mammalian cell lines and transgenic animals. *Mol Biotechnol* 34(3):337–354.

Redko, Y., Courtin, P., Mezange, C., Huard, C., Chapot-Chartier, M.P. 2007. *Lactococcus lactis* gene yjgB encodes a gamma-D-glutaminyl-L-lysyl-endopeptidase which hydrolyzes peptidoglycan. *Applied Environmental Microbiology* 73:5825–5831.

Reed, R. 2003. Coupling transcription, splicing and mRNA export. *Curr Opin Cell Biol*. ;15(3):326-31.

Rescigno, M., Valzasina, B., Bonasio, R., Urbano, M. and Ricciardi-Castagnoli, P. 2001. Dendritic cells, loaded with recombinant bacteria expressing tumor antigens, induce a protective tumor-specific response. *Clin Cancer Res*.; 7(3 Suppl):865s-870s.

Ribeiro, L. A., Azevedo, V., Le Loir, Y., Oliveira, S. C., Dieye, Y., Piard, J. C., Gruss, A. and Langella, P. 2002. Production and targeting of the *Brucella abortus* antigen L7/L12 in *Lactococcus lactis*: a first step towards food-grade live vaccines against brucellosis. *Appl. Environ. Microbiol*.(68) 2: 910-916.

Ribelles, P., Benbouziane, B., Langella, P., Suárez, J.E., Bermúdez-Humarán, L.G. 2013. Protection against human papillomavirus type 16-induced tumors in mice using non-genetically modified lactic acid bacteria displaying E7 antigen at its surface. *Appl Microbiol Biotechnol*. ;97(3):1395

Riley, J.P., Rosenberg, S.A. and Parkhurst, M.R. 2003. Stimulation of tumor-reactive T lymphocytes using mixtures of synthetic peptides derived from tumor-associated antigens with diverse MHC binding affinities. *J Immunol Methods*.; 276(1-2):103-19.

Robinson, K., Chamberlain, L. M., Lopez, M. C., Rush, C. M., Marcotte, H., Le Page, R. W. and Wells, J. M. 2004. Mucosal and cellular immune responses elicited by recombinant *Lactococcus lactis* strains expressing tetanus toxin fragment C. *Infect. Immun*. (72) 5: 2753-2761.

Robinson, K., Chamberlain, L. M., Schofield, K. M., Wells, J. M. and Le Page, R. W. 1997. Oral vaccination of mice against tetanus with recombinant *Lactococcus lactis*. *Nat. Biotechnol*. (15) 7: 653-657.

Rogers, H.J., Perkins, H.R. and Ward, J.B. 1980. *The bacterial autolysins. In Microbial Cell Walls and Membranes* (RogersHJ, PerkinsHR & WardJB, eds), pp. 437–460. Chapman and Hall, London.

Romanos, M. 1995. Advances in the use of *Pichia pastoris* for high-level gene expression. *Current Opinion in Biotechnology*, 6:527-533.

© COPYRIGHT UPM

Rout, M.P. and Aitchison, J.D. 2001. The nuclear pore complex as a transport machine. *J Biol Chem*.; 276(20):16593-6.

Russo, V., Cipponi, A., Raccosta, L., Rainelli, C., Fontana, R., Maggioni, D., Lunghi, F., Mukenge, S., Ciceri, F., Bregni, M., Bordignon, C. and Traversari, C. 2007. Lymphocytes genetically modified to express tumor antigens target DCs in vivo and induce antitumor immunity. *J Clin Invest*.; 117(10): 3087-96.

Saikali, S., Avril, T., Collet, B., Hamlat, A., Bansard, J.Y., Drenou, B., Guegan, Y. and Quillien, V. 2007. Expression of nine tumour antigens in a series of human glioblastoma multiforme: interest of EGFRvIII, IL-13Ralpha2, gp100 and TRP-2 for immunotherapy. *J Neurooncol*.; 81(2):139-48.

Samuelson, P., Gunneriusson, E., Nygren, P. A. and S. Stahl. 2002. Display of proteins on bacteria. *Journal of Biotechnology*., 96, (2): 129-54.

Sára M. 2001. Conserved anchoring mechanisms between crystalline cell surface S-layer proteins and secondary cell wall polymers in Gram-positive bacteria? *Trends Microbiology* 9: 47–49.

Satonaka, K., Ohashi, K., Nohmi, T., Yamamoto, T., Abe, S., Uchida, K. and Yamaguchi, H.1996. Prophylactic effect of *Enterococcus faecalis* FK-23 preparation on experimental candidiasis in mice. *Microbiology and Immunology*, 40, 217–222.

Schietinger, A., Philip, M. and Schreiber, H. 2008. Specificity in cancer immunotherapy. *Semin Immunol* ;20(5):276-85.

Schleifer, K. H., and Kandler, O. 1972. Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriology Review*. 36, 407–477.

Schreiber, H. and Paul W. 2003. Fundamental Immunology. 5th edition. Lippincott-Williams & Wilkins; p.p 1557-92.

Schubert, K., Bichlmaier, A.M., Mager, E., Wolff, K., Ruhland, G. and Fiedler, F. 2000. P45, an extracellular 45 kDa protein of *Listeria monocytogenes* with similarity to protein p60 and exhibiting peptidoglycan lytic activity. *Arch Microbiol* 173:21–28.

Schuster, M., Nechansky, A., Kircheis, R. 2006. Cancer immunotherapy. *Biotechnol. J*.1-138.

Sekiguchi, J., Akeo, K., Yamamoto, H., Khasanov, F.K., Alonso, J.C. and Kuroda, A. 1995. Nucleotide sequence and regulation of a new putative cell wall hydrolase gene, cwlD, which affects germination in *Bacillus subtilis*. *J Bacteriol* 177:5582–5589.

© COPYRIGHT UPM

Seo, N., Tokura, Y., Nishijima, T., Hashizume, H., Furukawa, F. and Takigawa, M. 2000. Percutaneous peptide immunization via corneum barrier-disrupted murine skin for experimental tumor immunoprophylaxis. *Proc Natl Acad Sci U S A.*; 97(1):371-6.

Shibagaki, N. and Udey, M.C. 2003. Dendritic cells transduced with TAT protein transduction domain-containing tyrosinase-related protein 2 vaccinate against murine melanoma. *Eur J Immunol*.; 33(4):850-60.

Shimizu, K., Thomas, E.K., Giedlin, M. and Mulé, J.J. 2001. Enhancement of tumor lysate- and peptide-pulsed dendritic cell-based vaccines by the addition of foreign helper protein. *Cancer Res.*; 61(6):2618-24.

Shockman, G.D. and Barrett, J.F. 1983. Structure, function, and assembly of cell walls of Gram-positive bacteria. *Annual Review of Microbiology*. 37: 501–527.

Şimşek, Ö., Sabanoğlu, S., Çon, A.H., Karasu, N., Akçelik, M. and Saris, P.E.J. 2013. Immobilization of nisin producer *Lactococcus lactis* strains to chitin with surface-displayed chitin-binding domain. *Appl Microbiol Biotechnol* 97:4577–4587.

Sin, J.I., Park, J.B., Lee, I.H., Park, D., Choi, Y.S., Choe, J. and Celis, E. 2012. Intratumoral electroporation of IL-12 cDNA eradicates established melanomas by Trp2(180-188)-specific CD8+ CTLs in a perforin/granzyme-mediated and IFN-γ-dependent manner: application of Trp2(180-188) peptides. *Cancer Immunol Immunother*.; 61(10):1671-82.

Sinha, B., Francois, P., Que, Y. A., Hussain, M., Heilmann, C., Moreillon, P., Lew, D., Krause, K. H., Peters, G. and Herrmann, M. 2000. Heterologously expressed *Staphylococcus aureus* fibronectin-binding proteins are sufficient for invasion of host cells. *Infect. Immun.* (68) 12: 6871-6878.

Slingluff, C.L. Jr., Petroni, G.R., Yamshchikov, G.V., Barnd, D.L., Eastham, S., Galavotti, H., Patterson, J.W., Deacon, D.H., Hibbitts, S., Teates, D., Neese, P.Y., Grosh, W.W., Chianese-Bullock, K.A., Woodson, E.M., Wiernasz, C.J., Merrill, P., Gibson, J., Ross, M., Engelhard, V.H. 2003. Clinical and immunologic results of a randomized phase II trial of vaccination using four melanoma peptides either administered in granulocyte-macrophage colony-stimulating factor in adjuvant or pulsed on dendritic cells. *J Clin Oncol*.; 21(21): 4016-26.

Solá, R.J. and Griebenow, K. 2009. Effects of glycosylation on the stability of protein pharmaceuticals. *J Pharm Sci*.; 98(4):1223-45.

© COPYRIGHT UPM

Solano, F., Jiménez-Cervantes, C., Martínez-Liarte, J.H., García-Borrón, J.C., Jara, J.R., Lozano, J.A. 1996. Molecular mechanism for catalysis by a new zinc-enzyme, dopachrome tautomerase. *Biochem J.* ;313 (Pt 2):447-53.

Song, D. and Gu, Q. 2009. Surface expression of *Helicobacter pylori* urease subunit B gene E fragment on *Lactococcus lactis* by means of the cell wall anchor of *Staphylococcus aureus* protein A. *Biotechnol. Lett*. (31) 7: 985-989.

Spiro, R. G. 2002. Protein glycosylation: Nature, distribution, enzymatic formation, and disease implications of glycopeptide bonds. *Glycobiology*. 12, 43R-56R.

Stahl, S. and Uhle´n, M., 1997. Bacterial surface display: trends and progress. *Trends Biotechnol*. 15, 185–192.

Stanley, P., Schachter, H. and Taniguchi, N. 2009. *N-Glycans. In: Essentials of Glycobiology*. 2nd edition. Cold Spring Harbor Laboratory Press. New York (USA).

Stapleton, M.R., Horsburgh, M.J., Hayhurst, E.J., Wright, L., Jonsson, I-M., Tarkowski, A., Kokai-Kun, J.F., Mond, J.J. and Foster, S.J. 2007. Characterization of IsaA and SceD, two putative lytic transglycosylases of *Staphylococcus aureus*. *Journal of Bacteriology* 189:7316–7325.

Steen, A., Buist, G., Leenhouts, K.J., El Khattabi, M., Grijpstra, F., Zomer, A.L., Venema, G., Kuipers, O.P. and Kok, J. 2003. Cell wall attachment of a widely distributed peptidoglycan binding domain is hindered by cell wall constituents. *Journal of Biology Chemistry*. 278:23874–23881.

Steidler, L., Robinson, K., Chamberlain, L., Schofi eld, K. M., Remaut, E., Le Page, R. W. and 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) 7: 3183-3189.

Steitz, J., Brück, J., Lenz, J., Knop, J. and Tüting T. 2001. Depletion of CD25(+) CD4(+) T cells and treatment with tyrosinase-related protein 2-transduced dendritic cells enhance the interferon alpha-induced, CD8(+) T-cell-dependent immune defense of B16 melanoma. *Cancer Res*.; 61(24):8643-6.

Steitz, J., Brück, J., Steinbrink, K., Enk, A., Knop, J. and Tüting T. 2000. Genetic immunization of mice with human tyrosinase-related protein 2: implications for the immunotherapy of melanoma. *Int J Cancer*.; 86(1):89-94.

Stewart, R.S., Drisaldi, B., Harris, D.A. 2001. A transmembrane form of the prion protein contains an uncleaved signal peptide and is retained in the endoplasmic Reticulum. *Mol Biol Cell*.;12(4):881-9.

© COPYRIGHT UPM

Stratton-Thomas, J.R., Min, H.Y., Kaufman, S.E., Chiu, C.Y., Mullenbach, G.T. and Rosenberg, S. 1995. Yeast expression and phagemid display of the human urokinase plasminogen activator epidermal growth factor-like domain. *Protein Engineering* ;8(5):463-70.

Sugai, M., Komatsuzawa, H., Akiyama, T., Hong, Y-M., Oshida, T., Miyake, Y., Yamaguchi, T., Suginawa, H. 1995. Identification of endo-beta-N-acetylglucosaminidase and N-acetylmuramyl-L-alanine amidase as cluster-dispersing enzymes in *Staphylococcus aureus*. *Journal of Bacteriology* 177:1491–1496.

Sullivan J.J., Jago G.R. and Mou L. 1976. Autolysis of Streptococcus cremoris. *Journal of Dairy Research* ;43(2):275-82.

Swanton, E. and Bulleid, N.J. 2003. Protein folding and translocation across the endoplasmic reticulum membrane. *Mol Membr Biol*.; 20(2):99-104.

Takakura Y., Takemoto S., Nishikawa, M. 2007. Hsp-based tumor vaccines: state-of-the-art and future directions, *Curr. Opin. Mol. Ther*. 9 385 ;9(4):385-91.

Tarahomjoo, S., Katakura, Y., Satoh, E. and Shioya, S. 2008. Expression of C-terminal repeat region of peptidoglycan hydrolase of *Lactococcus lactis* IL1403 in methylotrophic yeast *Pichia pastoris*. *J Biosci Bioeng* ;105(2):134-9.

Tjio, J. H., and Puck. T. T. 1958. Genetics of somatic mammalian cells. II. Chromosomal constitution of cells in tissue culture. *J. Exp. Med.*, 108(2), pp. 259–268.

Ton-That, H., Faull, K.F. and Schneewind, O. 1997. Anchor structure of staphylococcal surface proteins. A branched peptide that links the carboxyl terminus of proteins to the cell wall. *Journal of Biology and Chemistry*. 272, 22285–22292.

Ton-That, H., Marraffini, L.A. and Schneewind, O. 2004. Protein sorting to the cell wall envelope of Gram-positive bacteria. *Biochimica et Biophysica Acta* 1694 (2004) 269 – 278.

Tsukamoto K., Jackson I-J., Urabe K., Montague P.M. and Hearing V.J. 1992. A second tyrosinase-related protein, TRP-2, is a melanogenic enzyme termed DOPAchrome tautomerase. *The EMBO journal* vol.11 no.2 pp. 519-526.

Turner, M. S., Timms, P., Hafner, L. M. and Giffard, P. M. 1997. Identification and characterization of a basic cell surface-located protein from *Lactobacillus fermentum* BR11. *J Bacteriol* 179, 3310–3316.

Tüting, T., Steitz, J., Brück, J., Gambotto, A., Steinbrink, K., DeLeo, A.B., Robbins, P., Knop, J. and Enk, A.H. 1999. Dendritic cell-based genetic immunization in

© COPYRIGHT UPM

mice with a recombinant adenovirus encoding murine TRP2 induces effective anti-melanoma immunity. *J Gene Med.*; 1(6):400-6.

van Vliet, C., Thomas, E.C., Merino-Trigo, A., Teasdale, R.D. and Gleeson, P.A. 2003. Intracellular sorting and transport of proteins. *Prog Biophys Mol Biol*.; 83(1):1-45.

Varma, N.R.S, Toosa, H., Foo, H.L., Alitheen, N.B.M., Nor Shamsudin, M., Arbab, A.S, Yusoff, K., Abdul Rahim, R. 2013. Display of the viral epitopes on *Lactococcus lactis*: a model for food grade vaccine against EV71. *Biotechnol Res Int* :431315.

Vavricka, C.J., Ray, K.W., Christensen, B.M. and Li, J. 2010. Purification and N-glycosylation analysis of melanoma antigen dopachrome tautomerase. *Protein J.*; 29(3):204-12.

Vedeler, A., Pryme, I.F. and Hesketh, J.E. 1991. The characterization of free, cytoskeletal and membrane-bound polysomes in Krebs II ascites and 3T3 cells. *Mol Cell Biochem*.; 100(2):183-93.

Vos, P., Simons, G., Siezen, R.J., de Vos, W.M. 1989. Primary structure and organization of the gene for a prokaryotic, cell envelope-located serine proteinase. *J. Biol. Chem*. 264: 13579-13585.

Waligora, A.J., Hennequin, C., Mullany, P., Bourlioux, P., Collignon, A. and Karjalainen T. 2001. Characterization of a cell surface protein of *Clostridium difficile* with adhesive properties. *Infection Immunology* ;69(4):2144-53.

Walsh, C. 2006. *Posttranslational modification of proteins: Expanding nature's inventory*. Englewood, Colo.: Roberts and Co. Publishers. xxi, 490 p. p.

Walter, P. and Johnson, A.E. 1994. Signal sequence recognition and protein targeting to the endoplasmic reticulum membrane. *Annu Rev Cell Biol*.; 10:87-119.

Wang, L. and Lin, M. 2007 Identification of IspC, an 86-kilodalton protein target of humoral immune response to infection with *Listeria monocytogenes* serotype 4b, as a novel surface autolysin. *Journal of Bacteriology* 189:2046–2054.

Wang, X.Y., Sun, X., Chen, X., Facciponte, J., Repasky, E.A., Kane, J. and Subjeck, J.R. 2010. Superior antitumor response induced by large stress protein chaperoned protein antigen compared with peptide antigen. *J Immunol*.; 184(11):6309-19.

Wegmann, U., O'Connell-Motherway, M., Zomer, A., Buist, G., Shearman, C., Canchaya, C., Ventura, M., Goesmann, A., Gasson, M. J., Kuipers, O. P., van Sinderen, D. and Kok, J. 2007. Complete genome sequence of the prototype

© COPYRIGHT UPM

lactic acid bacterium *Lactococcus lactis* subsp. Cremoris. MG1363. *J. Bacteriol.* (189) 8: 3256-3270.

Wuenscher, M.D., Kohler, S., Bubert, A., Gerike, U. and Goebel, W. 1993. The iap gene of *Listeria monocytogenes* is essential for cell viability, and its gene product, p60, has bacteriolytic activity. *Journal of Bacteriology* 175:3491–3501.

Wurm, F.M. 2004. Production of recombinant protein therapeutics in cultivated mammalian cells. *Nat Biotechnol* 22(11):1393–1398.

Xu, G., Smith, T., Grey, F., and Hill, A.B. 2013. Cytomegalovirus-based cancer vaccines expressing TRP2 induce rejection of melanoma in mice. *Biochem Biophys Res Commun*.; 437(2):287-91.

Xu, Z., Ramishetti, S., Tseng, Y.C., Guo, S., Wang, Y. and Huang, L. 2013. Multifunctional nanoparticles co-delivering Trp2 peptide and CpG adjuvant induce potent cytotoxic T-lymphocyte response against melanoma and its lung metastasis. *J Control Release*.; 172 (1):259-65.

Yamaguchi, H., Furuhata, K., Fukushima, T., Yamamoto, H. and Sekiguchi, J. 2004. Characterization of a new *Bacillus subtilis* peptidoglycan hydrolase gene, yvcE (named cwlO), and the enzymatic properties of its encoded protein. *J Biosci Bioeng* 98:174–181.

Yamano, T., Kaneda, Y., Sharon, H., Hiramatsu, H.S., and S.B. Hoon, D., 2005. Enhancement of Immunity by a DNA melanoma vaccine against TRP2 with CCL21 as an adjuvant. *Molecular Therapy* Vol. 13.

Yu, Z., Theoret, M.R., Touloukian, C.E., Surman, D.R, Garman, S.C, Feigenbaum L, Baxter, T.K., Baker, B.M. and Restifo, N.P. 2004. Poor immunogenicity of a self/tumor antigen derives from peptide-MHC-I instability and is independent of tolerance. *J Clin Invest*; 114(4):551–9.

Yuan, R. and Hamilton, D.L. 1984. Type I and Type III Restriction-Modification Enzymes. *Springer Series in Molecular Biology 1984*, pp 11-37.

Raleigh, E.A. 1992. Organization and function of the mcrBC genes of *Escherichia coli* K-12. *Molecular Microbiology* 6, 1079–86.

Zhang, L., Leng, Q. and Mixson, A.J. 2005. Alteration in the IL-2 signal peptide affects secretion of proteins in vitro and in vivo. *J Gene Med*.; 7(3):354-65.

Zhang, M., Obata, C., Hisaeda, H., Ishii, K., Murata, S., Chiba, T., Tanaka, K., Li, Y., Furue, M., Chou, B., Imai, T., Duan, X. and Himeno, K. 2005. A novel

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DNA vaccine based on ubiquitin-proteasome pathway targeting 'self'-antigens expressed in melanoma/melanocyte. *Gene Ther*.; 12(13):1049-57.

Zhang, T.T., Kang, T.H., Ma, B., Xu, Y., Hung, C.F. and Wu, T.C. 2012. LAH4 enhances CD8+ T cell immunity of protein/peptide-based vaccines. *Vaccine*.; 30(4):784-93.

Zhu, X., Cai, J., Huang, J., Jiang, X., and Ren, D. 2010. The treatment and prevention of mouse melanoma with an oral DNA vaccine carried by attenuated *Salmonella typhimurium*. *Journal of Immunotheraphy* ;33(5):453-60.

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