UNIVERSITI PUTRA MALAYSIA

IDENTIFICATION OF A HEAT-SHOCK PROTEIN PROMOTER AND CONSTRUCTION OF A NOVEL STRESS INDUCIBLE LACTOBACILLUS-LACTOCOCCUS SHUTTLE VECTOR

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IDENTIFICATION OF A HEAT-SHOCK PROTEIN PROMOTER AND CONSTRUCTION OF A NOVEL STRESS INDUCIBLE *LACTOBACILLUS-LACTOCOCCUS* SHUTTLE VECTOR

By

MOHD SHAWAL THAKIB BIN MAIDIN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Master of Science

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Abstract of thesis presented to the senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Master of Science

IDENTIFICATION OF A HEAT SHOCK PROTEIN PROMOTER AND CONSTRUCTION OF A NOVEL STRESS INDUCIBLE *LACTOBACILLUS-LACTOCOCCUS* SHUTTLE VECTOR

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September 2013

Chairman: Prof. Raha Binti Abdul Rahim, PhD

Faculty: Biotechnology and Biomolecular Sciences

Lactic Acid Bacteria (LAB) have been long established as a consortium of bacteria with high economic value. Among the sub-species of LAB, *Lactobacillus plantarum* and *Lactococcus lactis* are the two most studied and widely commercialised. This includes their applications such as being used as starter culture in fermentation industries, vaccine development and metabolite production. In addition, these LABs have been genetically engineered because of their small genomes, easy genetic accessibility and the availability of genetic tools. However, there is still a lack of available shuttle vectors with strong promoter and broad host range for these two species of LAB. In addition, other promoters except for the nisin promoter are also less efficient. A new promoter inducible by stress may offer several advantages such as ease of gene expression, low cost and broad host range compatibility. In this study, bacteria containing plasmid was screened and identified as *Enterococcus faecium* HB6. The plasmid was characterised and named as pAR6. Three putative open reading frames (ORF) coding for the epsilon antitoxin, small heat shock protein

and replication were identified. The small heat shock protein promoter (P_{hsp}) was characterised and cloned into lactococcal promoter screening vector, pNZ8008. The quantitative expression analysis of P_{hsp} under various stress conditions showed that the maximum gusA (β-glucuronidase) activity was achieved under a stress combination of heat at 37°C, media with salt concentration of 3% (w/v) and alkalinity of pH9. A new *Lactobacillus-Lactococcus* shuttle vector pAR1801 was constructed by ligating the cassettes between a previously isolated *Lactobacillus* plasmid, pR18 containing the mob, sso, dso, repA and terminator sequence with *Lactococcus* pNZ8048-P_{hsp} vector, containing the chloramphenicol resistant gene, enterococcal P_{hsp} replaced lactococcal P_{nis}, multiple cloning site and terminator site. The shuttle vector was transformed into *Lb. plantarum* Pa21 and *L. lactis* NZ9000 hosts and was stably maintained until 50 generations. Results from relative qPCR determined that the copy number of the plasmid vector pAR1801 in *Lb. plantarum* Pa21 and *L. lactis* NZ9000 hosts were 34 and 31 copies per cell respectively. The functionality of pAR1801 to carry and express foreign genes was tested using the *gusA* gene. The pAR1801-GUS recombinant plasmid was transformed into both hosts, and was able to replicate and express the *gusA* gene as was shown by the ability of the cells to hydrolyse the X-Gluc (5-bromo-4-chloro-3-indolyl glucuronide). In conclusion, a hsp promoter was cloned and a functional stress inducible *Lactobacillus* and *Lactococcus* shuttle vector was constructed.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai Memenuhi keperluan untuk Ijazah Master Sains

MENGENALPASTI PROMOTER PROTIN KEJUTAN HABA DAN PEMBINAAN NOVEL VECTOR PEMBAWA KAWALAN STRESS *LACTOBACILLUS-LACTOCOCCUS*

Oleh

MOHD SHAWAL THAKIB MAIDIN

September 2013

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Bakteria Asid Laktik (LAB) telah lama wujud sebagai sebuah consortium bacteria dengan nilai ekonomi yang tinggi. Antara sub-spesies LAB, *Lactobacillus plantarum* dan *Lactococcus lactis* adalah dua sub-spesies yang paling dikaji dan dikomersilkan secara meluas. Ini termasuk aplikasi sebagai kultur pemula dalam industry penapaian, pembangunan vaksin dan pengeluaran metabolit. Di samping itu, pelbagai LAB telah melalui proses kejuruteraan genetic kerana genom mereka yang kecil, kemudahan untuk mendapatkan maklumat genetic dan ketersediaan alat genetik. Walaubagaimanapun, masih terdapat kekurangan bekalan vector pembawa dengan promoter yang kuat serta berkebolehan untuk melakukan replikasi di dalam pelbagai hos. Di samping itu, promoter lain kecuali promoter nisin juga kurang berkesan. Promoter daripada protein kejutan haba menawarkan beberapa kelebihan seperti memudahkan ekspresi gen, kos yang rendah dan keserasian di dalam pelbagai hos. Dalam kajian ini, bakteria yang mengandungi plasmid telah dicari dan dipencilkan. Hos LAB plasmid yang dicirikan sebagai pAR6 telah dikenal pasti

sebagai *Enterococcus faecium* HB6. Tiga bingkai putative bacaan terbuka (ORF) yang mengkod untuk antitoksin epsilon, haba kecil kejutan protein dan replikasi telah dikenal pasti. Haba protein kejutan kecil penganjur (P_{hsp}) promoter telah diklonkan ke dalam promoter saringan lactococcal vektor, pNZ8008. Ungkapan analisis kuantitatif P_{hsp} di bawah pelbagai keadaan tegasan menunjukkan bahawa aktiviti gusA maksimum (β-glucuronidase) telah dicapai di bawah kombinasi tekanan haba pada 37°C, media dengan kepekatan garam sebanyak 3% (w/v) dan kealkalian pH9 . *Lactobacillus-Lactococcus* vector pembawa pAR1801 yang baru telah dibina melalui ligasi kaset antara satu plasmid *Lactobacillus* yang sebelumnya terpencil, pR18 yang mengandungi SSO, DSO, RepS dan terminator dengan *Lactococcus* pNZ8048-P_{hsp}vector yang mengandungi gen tahan chloramphenicol, P_{nis}lactococcal digantikan oleh P_{hsp} enterococcal, pengklonan tapak pelbagai dan tapak terminator. Vektor pembawa telah dimasukkan ke dalam hos *Lb. plantarum* Pa21 dan *L. lactis* NZ9000 dan kestabilan telah dikekalkan sehingga 50 generasi.Keputusan dari qPCR relative menunjukkan bahawa bilangan salinan pAR1801 vektor plasmid dalam hos *Lb. plantarum* Pa21 dan *L. lactis* NZ9000 telah ditentukan sebagai 34 dan 31 salinan setiap sel masing-masing. Fungsi pAR1801 untuk menjalankan dan meluahkan gen asing telah diuji menggunakan gen *gusA*. Plasmid pAR1801-GUS rekombinan telah dimasukkan ke dalam kedua-dua hos, dan mampu bereplikasi dan mengekspres gen *gusA* seperti yang telah ditunjukkan oleh keupayaan sel-sel untuk hidrolisis X-Gluc (5-bromo-4-chloro-3-indolyl glucuronide). Kesimpulannya, promoter hsp telah diklon dan tekanan berfungsi teraruh *Lactobacillus* dan *Lactococcus* vector pembawa telah dibina.

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APPROVAL

I certify that an Examination committee has met on the date of viva voce to conduct the final examination of Mohd Shawal Thakib bin Maidin on his degree of Master of Science thesis entitled "Identification of a Heat Shock Protein Promoter and Construction of a Novel Stress Inducible *Lactobacillus-Lactococcus* Shuttle Vector.

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DECLARATION

I declare that the thesis is my original work except for quotations and citation which has been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Putra Malaysia or other institutions.

_____

MOHD SHAWAL THAKIB B. MAIDIN

Date: 2 December 2013

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LIST OF ABBREVIATIONS

~	approximately
A_{260}	Absorbance at 260 nm
a.a.	amino acids
Amp^r	ampicillin resistant
ATCC	American Type Culture Collection
B.C.	before century
BLAST	Basic Local Alignment Search Tool
bp	base pair
$CaCl_2$	calcium chloride
cat	chloramphenicol acetyltransferase
Chl^r	chloramphenicol resistant
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleotide triphosphate
DR	direct repeat
dsDNA	double-stranded DNA
dso	double strand origin
EDTA	ethylene diaminetetraacetic acid
Erm^r	Erythromycin resistant
FDA	Food and Drug Administration
G+C	guanine plus cytosine
g	gravity force
GM17	M17 supplemented with 0.5% glucose
GRAS	generally regarded as safe

gusA	β-glucuronidase
HSP	heat shock protein
HTH	αhelix-turn-αhelix
IR	inverted repeat
IS	insertion sequence
kb	kilo base pair
kDa	kilo Dalton
LAB	lactic acid bacteria
LB	Luria Bertani
M	Molar
MCS	multiple cloning site
$MgCl_2$	magnesium chloride
$MgSO_4$	magnesium sulphate
mRNA	messenger RNA
MW	molecular weight
NaCl	sodium chloride
NaOH	sodium hydroxide
NCBI	National Center for Biotechnology Information
NICE	Nisin-control gene expression
nt	nucleotide
OD_{600}	optical density at 600 nm
ORF	open reading frame
PCR	polymerase chain reaction
P_{hsp}	heat stress protein promoter
P_{nis}	nisin inducible promoter

PNPG	para-nitrophenyl-β-D-glucuronic acid
qPCR	quantitative PCR
RBS	ribosome binding site
RC	rolling circle
RCR	rolling circle replicating
RE	restriction enzyme
RNA	ribonucleic acid
rpm	revolution per minute
rRNA	ribosomal RNA
RT	Real Time
sdH_2O	sterile distilled water
SDS	sodium dodecyl sulphate
SGM17	GM17 containing 0.5 M sucrose
SGM17MC	SGM17 containing 20 mM $MgCl_2$ and 2 mM $CaCl_2$
SSB	single-stranded DNA binding protein
ssDNA	single-stranded DNA
ssi	single-stranded initiation
sso	Single strand origin
subsp.	subspecies
TAE	Tris-acetate-EDTA
T_m	melting temperature
U	unit
USA	United States
V	Volt
v/v	volume per volume

w/v	weight per volume
X-gal	5-bromo-4-chloro-3-indoyle-β-D-galactopyranoside
X-gluc	5-bromo-4-chloro-3-indolyl glucuronide

CHAPTER 1

INTRODUCTION

Lactobacillus plantarum and *Lactococcus lactis* are among the most studied strains of Lactic Acid Bacteria (LAB). It is a group of bacteria with a GRAS status by U.S food and drug administration (FDA). The probiotic and antimicrobial activity of these strains had been shown to be beneficial to our health. For example, they have been utilised for vaccine delivery and in pharmaceutical manufacturing (Zhu *et al*., 2009). In addition, LAB has the potential to be an important cell factory for the production of heterologous protein production compared to the widely used *Escherichia coli*. This is due to the safe use features of this "friendly bacteria".

Nevertheless, the application of LAB can be further broaden if the bacteria are more tolerant to less favourable conditions and react swiftly to stress conditions such as high temperature, high salt concentration and high and low pH. For example in the probiotic food (Schiffrin *et al*., 2001) and life vaccines (Mercenier *et al*., 2000) development, the requirement for sturdy LAB strains is important because they need to express specific functions in harsh environments such as in the digestive tract with pH range of 1.5 to 7.0 (Cotter and Hill, 2003) and in an industrial setting at higher temperature than their optimum 30ºC growth temperature (Batt, 1999). A pH range of 4.0 to 5.0 and 7.5 to 8.0 were reported to be the lower and higher limit respectively for *Lactococcus lactis* depending on the medium composition and strain (Sanchez *et al*., 2008; van Niel and Hahn-Hägerdal, 1999).

The emerging interest of the field of synthetic biology has led to the rapid development in improving the overall quality of these LABs as a cell factory, thus,

providing an alternative for the construction of the bacterial host with the desirable characteristics (Teusink *et al*., 2011). In this case, the improvement of the strains for the ability to produce the protein of interest under harsh conditions can be achieved by the introduction of an expression system with novel promoters. Promoters inducible by specific stress conditions will make the industrial productions of enzymes or fine chemicals cheaper as they will express genes without having to add any inducers. Furthermore, a shuttle vector has the ability to replicate in different bacterial hosts. This makes it possible to clone and express the same gene of interest between two bacterial species and therefore, increasing the understanding of gene functions between different bacterial hosts.

This study aims to search for new promoters and develop a new shuttle vector that can replicate and express heterologous proteins in both *Lactococcus* and *Lactobacillus*. Therefore, the objectives of the study are:

- To screen for LABs with candidate plasmids for the construction of a shuttle expression vector.
- To isolate and characterise the heat shock protein promoter from the identified plasmid.
- To construct a new *Lactobacillus plantarum* Pa21-*Lactococcus lactis* NZ9000 shuttle expression vector with the incorporated heat stress promoter.

REFERENCES

Abeles, A.L. (1986) P1 plasmid replication. Purification and DNA-binding activity of the replication protein RepA.*The Journal of Biological Chemistry*261: 3548-55.

Ahmed, T., Kanwal, R. and Ayub, N. (2006). Influence of temperature on growth pattern of *Lactococcus lactis*, *Streptococcus cremoris* and *Lactobacillus acidophilus* isolated from camel milk. *Biotechnology* 5: 481-486.

Ahrne, S.G. Molin.and Axelsson, L. (1992). Transformation of *Lactobacillus Reuteri* with electroporation: studies on the erythromycin resistance plasmid pLUL631. *Current Microbiology* 24: 199-205.

Ahrne, S., Nobaek, S., Jeppsson, B., Adlerberth, I., Wold, A.E. and Molin, G. (1998).The normal *Lactobacillus* flora of healthy human rectal and oral mucosa. *Journal of Applied Microbiology* 85:88-94.

Al Askari, G., Kahouadji, A., Khedid, K., Charof, R. and Mennane, Z. (2012). Screenings of Lactic Acid Bacteria Isolated from Dried Fruits and Study of Their Antibacterial Activity.*Middle-East Journal of Scientific Research* 11: 209-215.

Alegre, M. T., Rodriguez, M. C. and Mesas, J. M. (2004). Transformation of *Lactobacillus plantarum* by electroporation with in vitro modified plasmid DNA. *FEMS Microbiology Letter* 241: 73-77.

Araya, T., lshinashi, N., Shlmamura, S., Tanaka, T. and Takahashi, H. (1993). Genetic and molecular analysis of the rpoD gene from *Lactococcus lactis*. *Bioscience, Biotechnology and Biochemistry*57:88-92.

Aquilanti, L., Santarelli, S., Silvestri, G., Osimani, A., Petruzzelli, A. and Clementi, F.(2007). The microbial ecology of a typical Italian salami during its natural fermentation. *International Journal of Food Microbiology* 120:136-145

Åvall-Jääskeläinen, S., Kylä-Nikkilä, K., Kahala, M., Miikkulainen- Lahti, T. and Palva, A. (2002). Surface display of foreign epitopes on the *Lactobacillus brevis* S-layer. *Applied and Environmental Microbiology* 68:5943– 5951.

Axelsson, L., Lindstad, G. and Naterstad, K. (2003). Development of an inducible gene expression system for *Lactobacillus sakei*. *Letter in Applied Microbiology* 37: 115–120.

Aymerich, T., Martin, B., Garriga, M. and Hugas, M. (2003) Microbial quality and directPCR identification of lactic acid bacteria and non-pathogenic staphylococci from artisanal low-acid sausages. *Applied and Environmental Microbiology* 69:4583-4594.

Bahl, H., Müller, H., Behrens, S., Joseph, H. and Narberhaus, F. (1995). Expression of heat shock genes in *Clostridium acetobutylicum*. *FEMS Microbiology Review* 17:341-348.

Bardwell, J.C.A. and Craig,E.A. (1988). Ancient heat shock gene is dispensable. *Journal of Bacteriology* 170:2977–2983.

Batt, C.A. (1999). *Lactococcus*. Department of Food Science, Cornell University, USA.

Benachour, A., Frere, J.and Novel, G. (1995). pUCL287 plasmid from *Tetragenococcus halophila* (*Pediococcus halophilus*) ATCC33315 represents a new theta-type replicon family of lactic acid bacteria. *FEMS Microbiology Letters* 128: 167–175.

Benachour, A., Frere, J., Flahaut, S., Novel, G., and Auffray, Y. (1997). Molecular analysis of the replication region of the theta replicating plasmid pUCL287 from *Tetragenococcus* (*Pediococcus*) *halophilus* ATCC33315. *Molecular and General Genetics* 255: 504–513.

Benov, L. and Fridovich, I. (1995). Superoxide dismutase protects against heat shock in *Escherichia coli*. *Journal of Bacteriology* 177:3344–3346.

Bermudez-Humaran, L.G., Langella, P., Miyoshi, A.*et al*. (2002). Production of human papillomavirus type 16 E7 protein in *Lactococcus lactis. Applied and Environmental Microbiology* 68: 917-22.

Bhowmik, T., Fernandez, L. and Steele,J.L. (1993). Gene replacement in *Lactobacillus helveticus* CNRZ 32. *Journal of Bacteriology* 175: 6341-6344.

Blattner, F.R., Plunkett, G., Bloch, C.A.*et al*. (1997) The complete genome sequence of *Escherichia coli* K-12. *Science* 277: 1453-1474.

Bolotin, A., Wincker, P., Mauger, S., Jaillon, O., Malarme, K., Weissenbach, J., Ehrlich, S. D. and Sorokin, A. (2001). The complete genome sequence of the lactic acid bacterium *Lactococcus lactis* ssp. *lactis* IL1403. *Genome Research*. 11, 731–753

Bolotin, A., Quinquis, B., Renault, P. *et al*. (2004). Complete genome sequence and comparative analysis of the dairy bacterium *Streptococcus thermophilus*. *Nature Biotechnology* 22: 1554–1558.

Boorstein, W., Ziegelhoffer, T. andCraig,E.A. (1994). Molecular evolution of the HSP70 multigene family. *Journal of Molecular Evolution* 38: 1–17.

Bouia, A., Bringel,F., Frey, L., Kammerer, B.,Belarbi, A., Guyonvarch, A. and Hubert. J.C. (1989). Structural organization of pLP1, a cryptic plasmid from *Lactobacillus plantarum* CCM 1904. *Plasmid* 22: 185-192.

Braat, H., Rottiers, P., Hommes, D.W.*et al*. (2006) A phase I trial with transgenic

bacteria expressing interleukin-10 in Crohn's disease. *Clinical Gastroenterol and Hepatology* 4: 754-9.

Bringel, F., Frey, L. and Gubert, J.C. (1989). Characterization, cloning, curing and distribution in lactic acid bacteria of pLP1, a plasmid from *Lactobacillus plantarum* CCM 1904 and its use in shuttle vector construction. *Plasmid* 22: 193-202.

Broadbent, J.R. and Lin, C. (1999). Effect of heat shock or cold shock treatment on the resistance of *Lactococcus lactis*to freezing and lyophilization. *Cryobiology* 39: 88–102.

Bryan, E.M., Bae, T., Kleerebezem, H. and Dunny, G.M. (2000). Improved vectors for nisin-controlled expression in gram-positive bacteria. *Plasmid* 44:183–190.

Buchner, J. (1999). Hsp90 & Co.—a holding for folding. *Trends in Biochemical Sciences* 24:136–141

Buchner, J. (1996). Supervising the fold: Functional principles of molecular chaperones. *FASEB Journal* 10:10–19.

Bukau, B. and Horwich,A.L. (1998). The Hsp70 and Hsp60 chaperone machines. *Cell* 92:351–366.

Burgess, C., O'Connell-Motherway, M., Sybesma, W., Hugenholtz, J. and van Sinderen, D.(2004). Riboflavin production in *Lactococcus lactis*: potential for in situ production of vitamin-enriched foods. *Applied and Environmental Microbiology* 70:5769-5777.

Burgos, J.S., Ramirez, C., Tenorio, R., Sastre, I., Bullido, M.J. (2002). Influence of reagents formulation on real-time PCR parameters. *Molecular and Cellular Probes* 16: 257–260.

Buxton, D.R., Muck, R.E., Harrison, J.H. (2003). Silage science and technology, Series Agronomy, ASA-MSSA-SSSA, Madison, Wisconsin, USA. 32: 547-595

Capozzi, V., Arena, M.P., Crisetti, E., Spano, G. and Fiocco, D. (2011). The hsp 16 Gene of the Probiotic*Lactobacillus acidophilus* Is Differently Regulated by Salt, High Temperature and Acidic Stresses, as Revealed by Reverse Transcription Quantitative PCR (qRT-PCR) Analysis. *International Journal of Molecular Sciences* 12:5390-5405

Carvalho, S.M., Kuipers, O.P. and Neves, A.R. (2013). Environmental and nutritional factors that affect growth and metabolism of the pneumococcal serotype 2 strain D39 and its nonencapsulated derivative strain r6. *PLOS ONE* 8: e58492.

Caspers, G.J., Leunissen, J.A.M. and de Jong, W.W. (1995). The expanding small heat-shock protein family, and structure predictions of the conserved 'α crystallin domain'. *Journal of Molecular Evolution* 40:238–248

Chaillou, S., Bor, Y.C., Batt, C.A., Postma, P.W., and Pouwels, P.H. (1998). Molecular Cloning and Functional Expression in *Lactobacillus* 80 of xylT, Encoding the D-Xylose–H+ Symporter of *Lactobacillus brevis*. *Applied and Environmental Microbiology, 64*, 4720–4728.

Chuang, S.E. and Blattner,F.R. (1993). Characterization of twenty-six new heat shock genes of *Escherichia coli. Journal of Bacteriol* 175:5242–5252.

Cocconcelli, P.S., Gasson,M.J., . Morelli, L. and Bottazzi, V. (1991).Single-stranded DNA plasmid vector construction and cloning of*Bacillus stearothermophilus* alpha-amylase in *Lactobacillus*. *Research in Microbiology* 142: 643-652.

Cotter,P.D. and Hill, C. (2003).Surviving the Acid Test: Responses of Gram-Positive Bacteria to Low pH. *Microbiology and Molecular Biology Reviews* 67: 429–453.

Dao, M.L. and Ferretti, J.J. (1985). *Streptococcus-Escherichia coli*shuttle vector pSA3 and its use in cloning of streptococcal genes.*Applied and Environmental Microbiology* 49: 115-119.

Davidson, B.E., Kordias, N., Dobos, M. and Hillier,A.J. (1996). Genomic organization of lactic acid bacteria.*Antonie van Leeuwenhoek.* 70: 161–183.

Davidson, J.F., Whyte, B., Bissinger, P.H. and Schiestl, R.H. (1996). Oxidative stress is involved in heat-induced cell death in *Saccharomyces cerevisiae*. *Proceeding of the National Academy of Sciences* USA 14:5116–5121**.**

de Bruyne, K., Camu, N., De Vuyst, L. and Vandamme, P.(2009).*Lactobacillus fabifermentans*sp. *nov*. and*Lactobacillus cacaonum* sp. *nov*., isolated from Ghanaian cocoa fermentations. *International Journal of Systematic and Evolutionary Microbiology* 59:7-12.

Deprez, R.H.L., Fijnvandraat, A.C., Ruijter, J.M., Moorman, A.F.M. (2002). Sensitivity and accuracy of quantitative real-time polymerase chain reaction using SYBR green I depends on cDNA synthesis conditions. Analytical in Biochemistry 307: 63–69.

Derre, I., Rapoport, G. and Msadek, T. (1999). CtsR, a novel regulator of stress and heat shock response, controls clp and molecular chaperone gene expression in gram-positive bacteria.*Molecular Microbiology* 3l: 117-131.

Derre, I., Rapoport, G. and Msadek, T. (2000). The CtsR regulator of stress response is active as a dimer and specifically degraded in vivo at 37 degrees C. *Molecular Microbiology*38: 335-347.

De Jong, W.W., Caspers, G.J. and Leunissen. J.A.M. (1998). Genealogy of the α crystallin–small heat-shock protein superfamily. *International Journal Biology Macromolecul* 22:151–162

de Oliveira N.E., Abranches, J., Gaca, A.O., Laport, M.S. and Damaso, C.R., *et al*. (2011). clpB, a class III heat-shock gene regulated by CtsR, is involved in thermotolerance and virulence of *Enterococcus faecalis*. *Microbiology* 157: 656-65

de Ruyter, P.G., Kuipers, O.P., Beerthuyzen, M.M., Alen-Boerrigter, I., de Vos, W.M. (1996a). Functional analysis of promoters in the nisin gene cluster of *Lactococcus lactis*. *Journal of Bacteriology* 178:3434–3439.

de Ruyter, P.G., Kuipers, O.P. and de Vos, W.M. (1996b). Controlled gene expression systems for *Lactococcus lactis*with the food-grade inducer nisin. Applied and Environmental Microbiology 62:3662–3667.

de Ruyter, P.G.G.A., Kuipers, O.P., Meijer, W.C., de Vos, W.M. (1997).Food-grade controlledlysis of *Lactococcus lactis* for accelerated cheese ripening. *Nature Biotechnology* 15:976-979.

deVos,W. M. (1986). Gene cloning in lactic streptococci. *Netherlands Milk and Dairy Journal*40: 141–154.

de Vos, W.M. (1987). Gene cloning and expression in lactic streptococci. *FEMS Microbiology Rev*iew 46: 281–295.

de Vos, W.M.(1999). Safe and sustainable systems for food-grade fermentations by genetically modified lactic acid bacteria. *International Dairy Journal* in press.

de Vos, W.M.(1999). Gene expression systems for lactic acid bacteria. Current Opinion in Microbiology 2, 289 – 295.

de Vos, W.M., Kleerebezem, M. and Kuipers, O.P.(1997).Expression systems for industrial Gram-positive bacteria with low guanine and cytosine content. *Current Opinion in Biotechnology* 8:547-553.

de Vos, W.M. and Simons, G. (1994). Gene cloning and expression systems in Lactococci. In *Genetics and Biotechnology of Lactic Acid Bacteria*. Edited by Gasson, M.J. and de Vos, W.M. Oxford: Chapman and Hall 52-105.

Di Cagno, R., De Angelis, M., Limitone, A., Fox, P.F. and Gobbetti, M. (2006). Respons of *Lactobacillus helveticus* PR4 to heat stress during propagation in cheese whey with a gradient of decreasing temperatures. *Applied and EnvironmentalMicrobiology* 72: 4503–4514.

Di Cagno, R., Coda, R., De Angelis, M. and Gobbetti, M. (2012). Exploitation of vegetables and fruits through lactic acid fermentation. *Food Microbiology* 33:1–10.

Dintilhac, A., Alloing, G. Granadel, C. and Claverys,J.P**. (**1997). Competence and virulence of *Streptococcus pneumoniae*: Adc and PsaA mutants exhibit a requirement for Zn and Mn resulting from inactivation of putative ABC metal permeases. *Molecular Microbiology* 25**:**727–739.

Dintilhac, A., and Claverys,J.P. (1997). The *adc*locus, which affects competence for genetic transformation in *Streptococcus pneumoniae*, encodes an ABC transporter with a putative lipoprotein homologous to a family of streptococcal adhesins. *Research Microbiology*148**:**119–131.

Dunne, C., Murphy, L., Flynn, S., O'Mahony, L., O'Halloran, S., Feeney, M., Morrissey, D., Thornton, G., Fitzgerald, G., Daly, C., Kiely, B., Quigley, E.M., O'Sullivan, G.C., Shanahan, F. and Collins, J.K. (1999). Probiotics: from myth to reality. Demonstration of functionality in animal models of disease and in human clinical trials. *Antonie Van Leeuwenhoek* 76: 279–292.

Dunning, A.M., Talmud, P. and Humphries, S.E. (1988). Errors in the polymerase chain reaction. *Nucleic Acids Research* 16: 10393.

Edwards, U., Rogall, T., Blöcker, H., Emde, M. and Böttger, E.C. (1989). Isolation and direct complete nucleotide determination of entire genes. Characterisation of a gene coding for 16S ribosomal RNA. *Nucleic Acids Research* 17: 7843-7853.

Eichenbaum, Z., Federle, M.J., Marra, D., de Vos, W.M., Kuipers, O.P., Kleerebezem, M. and Scott, J.R. (1998). Use of the lactococcal nisA promoter to regulate gene expression in gram-positive bacteria: comparison of induction level and promoter strength. *Applied and Environmental Microbiology* 64:2763–2769.

El Demerdash, H.A., Heller, K.J. and Geis, A. (2003). Application of the shsp gene, encoding a small heat shock protein, as a food-grade selection marker for lactic acid bacteria. *Applied and Environmental Microbiology*69: 4408-4412.

El Demerdash, H.A., Oxmann, J., Heller, K.J. and Geis, A. (2006). Yoghurt fermentation at elevated temperatures by strains of *Streptococcus thermophilus* expressing a small heat-shock protein: application of a two-plasmid system for constructing food-grade strains of *Streptococcus thermophilus*, *Biotechnology Journal*, Vol. 1, No. 4, pp. 398-404.

Ercolini, D., Hill, P.J. and Dodd, C.E. (2003) Bacterial community structure and location in Stilton cheese. *Applied and Environmental Microbiology* 69:3540-3548.

Elsholz, A.K., Michalik, S., Zuhlke, D., Hecker, M. and Gerth, U. (2010). CtsR, the Gram-positive master regulator of protein quality control, feels the heat. *TheEMBO Journal* 29: 3621-3629.

Elsholz, A.K., Hempel, K., Pother, D.C., Becher, D., Hecker, M. and Gerth, U.

(2011). CtsR inactivation during thiol-specific stress in low GC, Gram+ bacteria. *Molecular Microbiology* 79: 772-785.

Eskandari, H., Ghanbari, A. and Javanmard, A. (2009). Intercropping of cereals and legumes for forage production. *Notulae Scientia Biologicae* 1: 7-13.

Fayet, O., Ziegelhoffer, T. and Georgopolous,C. (1989). The groES and groEL heat shock gene products of *Escherichia coli* are essential for bacterial growth at all temperatures. *Journal of Bacteriology* 171: 1379–1385.

Fiocco, D., Capozzi, V., Collins, M., Gallone, A., Hols, P., Guzzo, J ., Weidmann, S., Rieu, A., Msadek, T. and Spano, G. (2009a). Characterization of the CtsR stress response regulon in *Lactobacillus plantarum. Journal of Bacteriology* 192: 896-900.

Fiocco, D., Collins, M., Muscariello, L., Hols, P., Kleerebezem, M., Msadek, T. and Spano, G. (2009b). The *Lactobacillus plantarum* ftsH gene is a novel member of the CtsR stress response regulon. *Journal of Bacteriology* 191: 1688-1694.

Friehs, K. (2004). Plasmid copy number and plasmid stability. *Advances in Biochemical Engineering / Biotechnology* 86:47–82.

Fujimura, H., Sakuma, Y., Weimer, T. (1996). A quantitative method for the determination of plasmid copy number in recombinant yeast. *Biologicals* 24: 67–70.

Ganzle, M.G., Vermeulen, N. and Vogel, R.F.(2007)Carbohydrate, peptide and lipidmetabolism of lactic acid bacteria in sourdough. *Food Microbiology*2:128-138.

Gardiner, G.E., O'Sullivan, E., Kelly, J., Auty, M.A.E., Fitzgerald, G.F., Collins, J.K., Ross, R.P.and Stanton, C. (2000). Comparative survival rates of human-derived probiotic *Lactobacillus paracasei* and *L. salivarius* strains during heattreatment and spray drying. *Applied and Environmetal Microbiology* 66:2605–2612.

Gasson, M.J. (1983). Plasmid complements of *Streptococcus lactis* NCDO712 and other lactic streptococci after protoplast-induced curing. *Journal of Bacteriology*154: 1-9.

Goloubinoff, P., Mogk, A., Peres Ben-Zvi, A., Tomoyasu, T. and Bukau, B. (1999). Sequential mechanism of solubilization and refolding of stable protein aggregates by a bichaperone network. Proceeding of the National Academy of Sciences USA 96:13732–13737.

Gruss, A. and Ehrlich, S.D. (1989). The family of highly interrelated single-stranded deoxyribonucleic acid plasmids. *Microbiology Review* 53:231–241.

Hammes, W.P. and Hertel, C. (2003). The Genera Lactobacillus and

Carnobacterium. In: *The Prokaryotes* Release 3.15, Editor Martin Dworkin

Han, M.J., Yun, H. and Lee, S.Y. (2008). Microbial small heat shock proteins and their use in biotechnology. *Biotechnology Advance* 26: 591-609.

Hanniffy, S.B., Carter, A.T., Hitchin, E. and Wells, J.M. (2007) Mucosal delivery of a pneumococcal vaccine using *Lactococcus lactis* affords protection against respiratory infection. *Journal of Infectious Diseases* 195: 185-93.

Hantke, K.**(**2001). Bacterial zinc transporters and regulators. *Biometals*14**:** 239–249.

Hashiba, H., Takiguchi , R., Ishii, S., and Aoyama, K. (1990). Transformation of *Lactobacillus helveticus* subsp. *jugurti* with plasmid pLHR by electroporation. *Agriculture and Biological Chemistry* 54: 1537-1541.

Hasper, H.E., de Kruijff, B. and Breukink, E.(2004) Assembly and stability of nisin Lipid II Pores. *Biochemistry*43:11567-11575.

Hayes, F., Daly, C., and Fitzgerald, G.F. (1990). Identification of the minimal replicon of *Lactococcuslactis* subsp.*lactis* UC317 plasmid pCI305. *Applied andEnvironmental Microbiology*56: 202–209.

Hayes, F., Vos, P., Fitzgerald, G.F., de Vos, W.M. and Daly, C. (1991). Molecular organization of the minimal replicon of novel, narrow host-range, lactococcal plasmid pCI305. *Plasmid*. 25: 16–26.

Hazlett, K.R., Rusnak, F.O., Kehres, G.D., Bearden,W.S., La Vake, J.C., M. E. La Vake, E.M., Maguire, Perry, D.R. and Radolf, D.J.(2003). The*Treponema pallidum tro* operon encodes a multiple metal transporter, azinc-dependent transcriptional repressor, and a semi-autonomously expressedphosphoglycerate mutase. *The Journal of Biology Chemistry*278**:**20687–20694.

Herman, C., Thevenet, D., D'ari, R. and Bouloc, P. (1995). Degradation of sigma 32, the heat shock regulator in *Escherichia coli*, is governed by HflB. *Proceedings of the National Academy of Sciences USA* 92: 3516 3520.

Hickey, R.M., Ross, R.P. and Hill, C.(2004). Controlled autolysis and enzyme release in a recombinant lactococcal strain expressing the metalloendopeptidase enterolysin A. *Applied and Environmental Microbiology*70:1744-1748.

Holo, H. and Nes, I.F. (1989) High-frequency transformation, by electroporation, of *Lactococcus lactis* subsp. *cremoris* grown with glycine in osmotically stabilized media. *Applied and Environmental Microbiology* 55: 3119-3123.

Hols, P., Ferain, T., Garmyn, D., Bernard, N. and Delcour, J. (1994). Use of homologous expression-secretion signals and vector-free stable chromosomal integration in engineering of *Lactobacillus plantarum*for α-amylase and

levanase expression. *Applied and Environmental Microbiology* 60:1401-1413.

Holt, Stephen, M.D., Linda, C.(1998). Miracle Herbs: How Herbs Combine with Modern Medicine to Treat Cancer, Heart Disease, AIDS, and More, Caro Publishing Group.

Ingmer, H. and Brondsted, L. (2009). Proteases in bacterial pathogenesis. *Research in Microbiology* 160: 704-10.

Jakob, U., Muse, W., Eser, M. and Bardwell,J.C.A. (1999). Chaperone activity with a redox switch. Cell 96:341–352.

Jakob, U., Gaestel, M., Engel, K. and Buchner, J. (1993). Small heat shock proteins are molecular chaperones. *Journal of Biological Chemistry* 268:1517–1520.

Jakubovics, N.S., Smith, W.A. and Jenkinson,F.H. (2000). Expression of the virulence-related Sca Mn2_ permease in *Streptococcus gordonii* is regulated by diphteria toxin metallorepressor-like protein ScaR. Molecular Microbiology. 38**:**140–153.

Janulczyk, R., Pallon,J. and Bjorck.L. (1999). Identification and characterization of a*Streptococcus pyogenes* ABC transporter with multiple specificity for metal cations. *Molecular Microbiology*34**:**596–606.

Jensen, P.R. and Hammer, K. (1998) Thesequence of spacers between the consensus sequences modulates the strength of prokaryotic promoters. *Applied and Environmental Microbiology*63:82-87.

Jensen, P.R., Westerhoff, H.V. and Michelsen, O. (1993). The use of lac-type promoters in control analysis. *European Journal of Biochemistry* 211: 181–191.

Jones, M.L., Chen, H., Ouyang, W., Metz, T. and Prakash, S. (2004) Microencapsulated genetically engineered *Lactobacillus plantarum*80 (pCBH1) acid deconjugation and its implication in loweringcholesterol. *Journal of Biomedicine Biotechnology* 1:61-69

Jung, C. M., Heinze, T. M., Schnakenberg, L. K., Mullis, L. B., Elkins, S. A., Elkins, C. A., Steel, R. S., and Sutherland,J. B. (2009). Interaction of Dietary Resveratrol with Animal-Associated Bacteria. *FEMS* 297: 266-273.

Kamble, R.D. and Pathade, G.R. (2010). Studies on potential application of representative promising isolates of *Lactobacillus*for preparation of soft drink-like lassie. *International Journal of Advanced Biotechnology and Research* 1: 5-10.

Khan, S. A.**(**1997). Rolling-circle replication of bacterial plasmids. *Microbiology andMolecular Biology Review*61**:**442–455.

Kiewiet, K., Kok, J., Seegers, J. F.M.L., Venema, G., and Bron, S. (1993). The mode of replication is a major factor in segregational plasmid instability in *Lactococcus lactis.Applied and Environmental Microbiology* 59: 358-364.

Kiewiet, R., Sierd, B., de Jonge, K., Venema, G. and Seegers, J.F.M.L. (1993). Theta replication of the lactococcal plasmid pWV02. *Molecular Microbiology* 10: 319–327.

Kilstrup, M and Hammer, K. (2000). Short communication: salt extends the upper temperature limit for growth of *Lactococcus lactis* ssp. *cremoris* on solid M17 medium. *Journal of Dairy Science* 83:1448–1450

Kim, S.F., Baek, S.J. and Pack, M.Y. (1991). Cloning and nucleotide sequence of the *Lactobacillus casei*lactate dehydrogenase gene. *Applied and Environmental Microbiology* 57: 2413–2417.

Kim, S.J., Jeong, D.G., Chi, S.W., Lee, J.S. and Ryu,S.E. (2001). Crystal structure of proteolytic fragments of the redox-sensitive Hsp33 with constitutive chaperone activity. *Nature Structural anf Molecular Biology* 8:459–466.

Kirstein, J., Schlothauer, T., Dougan, D.A., Lilie, H., Tischendorf, G., Mogk, A., Bukau, B. and Turgay, K. (2006). Adaptor protein controlled oligomerization activates the AAA+ protein ClpC. *TheEMBO Journal* 25: 1481-1491.

Kleerebezem, M., Beerthuyzen, M.M., Vaughan, E.E., de Vos, W.M. and Kuipers, O.P. (1997). Controlled gene expression systems for lactic acid bacteria: transferable nisin-inducible expression cassettes for *Lactococcus*, *Leuconostoc*, and *Lactobacillus* spp. *Applied and Environmental Microbiology* 63:4581–4584.

Kleerebezem, M., de Vos, W.M. and Kuipers, O.P. (1998). The lantibiotics nisin and subtilin act as extracellular regulators of their own biosynthesis. In: Dunny, G.M. and Winans, S.C. (eds) Cell–cell signalling in bacteria. *American Society for Microbiology* 159-173.

Klein, D. (2002). Quantification using real-time PCR technology: applications and limitations. *Trends in Molecular Medicine* 8: 257–260.

Klein, J.R., Ulrich, C. and Plapp,..R. (1993). Characterization and sequence analysis of a small cryptic plasmid from *Lactobacillus curvatus* LTH683 and its use for construction of new *Lactobacillus*cloning vecters. *Plasmid* 30: 14-29.

Klijn, N., Weerkamp, A.H. and de Vos, W.M. (1991). Identification of mesophilic lactic acid bacteria by using polymerase chain reaction-amplified variable regions of 16S rRNA and specific DNA probes. *Applied and Environmental Microbiology* 57:3390-3393.

Kojic, M., Lozo, J., Jovcic, B., Strahinic, I., Fira, D. and Topisirovic, L. (2010) A

successful use of a new shuttle cloning vector Pa13 for the cloning of the bacteriocins BACSJ and ACIDOCIN 8912. *Archives of Biological Sciences* 62: 231-243.

Kok, J., van der Vossen, J.M.B.M. and Venema. G. (1984). Construction of plasmid cloning vectors for lactic streptococci which also replicate in *Bacillus subtilis* and *Escherichia coli*. *Applied and Environmental Microbiology*48: 726-731.

Korpela, K., Buchert, P., Soderlund, H. (1987). Determination of plasmid copy number with nucleic acid sandwich hybridization. *Journal of Biotechnology* 5: 267–277.

Kruger, E. and Hecker, M. (1998). The first gene of the *Bacillus subtilis* clpC operon, ctsR, encodes a negative regulator of its own operon and other class III heat shock genes.*Journal of Bacteriology* 180: 6681-6688.

Kullen, M.J. and Klaenhammer, T.R. (1999). Genetic Modification of Intestinal Lactobacilli and Bifidobacteria. In: Tannock GW (Ed.) *Probiotics, a critical review*. Horizon Scientific Press, Wymondham 65–83.

Kunji, E.R.S., Slotboom, D.J. and Poolman, B. (2003). *Lactococcus lactis* as host for overproduction of functional membrane proteins. *Biochimica et Biophysica Acta* 1610: 97-108.

Kuipers, O.P., Beerthuyzen, M.M., de Ruyter, P.G.G.A., Luesink, E.J. and de Vos, W.M. (1995) Autoregulation of nisin biosynthesis in *Lactococcus lactis*by signal transduction. *Journal of Biological Chemistry* 270:27299-27304.

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

Lal, G., Siddappa, G.S. and Tandon, G.L. (1986), *Preservation of Fruits and Vegetables*, Indian Council of Agricultural Research, India.

Lee, K., Rho, B.S., Pi, K., Kim, H.J. and Choi, Y.J. (2011) Proteomic analysis of protein expression in *Lactobacillus plantarum* in response to alkaline stress. *Journal of Biotechnoogy153*: 1–7.

Lee, S., Kim, J., Shin, S. G., and Hwang, S. (2005) Absolute and relative QPCR quantification of plasmid copy number in *Escherichia coli*. *Journal of Biotechnology* 123: 273-280.

Lee, S.Y., Prochaska, D.J., Fang, F. and. Barnum, S.R. (1998). A 16.6- kilodalton protein in the cyanobacterium Synechocystis sp. PCC 6803 plays a role in the heat shock response. *Current Microbiology* 37:403–407.

Lehmann, U. Kreipe, H. (2001). Real-time PCR analysis of DNA and RNA extracted from formalin-fixed and paraffin-embedded biopsies. *Methods* 25: 409–418.

Leenhouts, K.J., Tolner, B., Bron, S., Kok, J., Venema, G. and Seegers, J.F. (1991). Nucleotide sequence and characterization of the broad host-range lactococcal plasmid pWV01. *Plasmid* 26:55–66.

Li, M. and. Wong,S.L (1992). Cloning and characterization of the groESL operon from *Bacillus subtilis*. *Journal of Bacteriology* 174:3981–3992

Li, R., Zhai, Z., Yin, S., Huang, Y., Wang, Q., Luo, Y., Hao, Y. (2009). Characterization of a rolling-circle replication plasmid pLR1 from *Lactobacillus plantarum* LR1. *Current Microbiology* 58: 106–110.

Linares, D.M., Kok, J., and Poolman, B. (2010). Genome Sequences of *Lactococcus lactis* MG1363 (Revised) and NZ9000 and Comparative Physiological Studies. *Journal of Bacteriology*192: 5806–5812.

Lindholm, A., Smeds, A. and Palva, A. (2004). Receptor binding domain of *Escherichia coli* F18 fimbrial adhesin FedF can be both efficiently secreted and surface displayed in a functional form in *Lactococcus lactis*. *Applied and Environmental Microbiology* 70:2061–2071

Liu, S.Q.(2002). A review: malolactic fermentation in wine -beyond deacidification. *Journal of Applied Microbiology* 92: 589-601.

Livak, K.J., Schmittgen, T.D. (2001). Analysis of relative gene expression data using real time quantitative PCR and the $2^{-\Delta\Delta CT}$method. *Methods* 25: 402–408.

Llull,D., Poquet, I. (2004). New expression system tightly controlled by zinc availability in *Lactococcus lactis. Applied and Environmental Microbiology* 70: 5398–5406

Lopez de Felipe, F., Kleerebezem, M., de Vos, W.M. and Hugenholtz, J. (1998). Cofactor engineering: a novel approach to metabolic engineering in *Lactococcus lactis* by controlled expression of NADH oxidase. *Journal of Bacteriology* 180: 3804–3808.

Luchansky, J.B., Muriana, P.M. and Klaenhammer, T.R. (1988). Application of electroporation for transfer of plasmid DNA to *Lactobacillus, Lactococcus, Leuconostoc, Listeria, Pediococcus, Bacillus, Staphylococcus, Enterococcus*, and *Propionibacterium*. *Molecular Microbiology* 2: 637-646.

Maassena, C., Laman, J., Bak-Glashouwer, M.H., Tielen, F., Holten-Neelen, J.V., Hoogteijlinga, L., *et al*. (1999). Instruments for oral disease-intervention strategies: recombinant *Lactobacillus casei* expressing tetanus toxin fragment C for vaccination or myelin proteins for oral tolerance induction in multiple sclerosis. *Vaccines* 17: 2117- 2128.

Madsen, S.M., Arnau, J., Vrang, A., *et al*. (1999).Molecular characterization of the pH-inducible and growth phasedependentpromoter P170 of *Lactococcus lactis*. *Molecular Microbiology* 32: 75-87.

Madsen, S.M., Hindre, T., Le Pennec, J.P., Israelsen, H. and Dufour,A. (2005) Two acid-inducible promoters from *Lactococcus lactis* require the cis-acting ACiD-box and the transcription regulator RcfB. *Molecular Microbiology* 56: 735–746.

McDuffee, A.T., Senisterra, G., Huntley, S., Lepock, J.R., Sekhar, K.R.,Meredith, M.J., Borrelli, M.J., Morrow, J.D. and Freeman. M.L. (1997). Proteins containing non-native disulfide bonds generated by oxidative stress can act as signals for the induction of the heat shock response. *Journal of Cellular Physiology* 171:143–151.

Meinhart, A., Alonso, J.C., Sträter, N., Saenger, W. (2003). Crystal structure of the plasmid maintenance system epsilon/zeta: functional mechanism of toxin zeta and inactivation by epsilon 2 zeta 2 complex formation. *Proceedings of the National Academy Sciences* 100:1661-1666.

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

Mierau, I., Kleerebezem, M., (2005). 10 years of the nisin-controlled gene expression system (NICE) in *Lactococcus lactis*. *Applied Microbiology and Biotechnology* 68: 705–717

Mills, S., McAuliffe, O.E., Coffey, A., Fitzgerald, G.F., Ross, R.P. (2006). Plasmids of lactococci - genetic accessories or genetic necessities? *FEMS Microbiology Reviews*30: 243-73.

Mierau, I. and Kleerebezem, M.(2005) 10 years of the nisin-controlled gene expression system (NICE) in *Lactococcus lactis*. *Applied Microbiology and Biotechnology* 68:705-717.

Molenaar, D., Bringel, F., Schuren, F.H., de Vos, W.M., Siezen, R.J. and Kleerebezem, M. (2005). Exploring *Lactobacillus plantarum*genome diversity by using microarrays. *Journal of Bacteriology* 187:6119‑6127

Morello, E., Bermudez-Humaran, L.G., Llull, D., Sole, V., Miraglio, N., Langella, P. andPoquet, I. (2008).*Lactococcus lactis*, an efficient cell factory for recombinant protein production and secretion. *Journal of Molecular Microbiology and Biotechnology* 14:48-58.

Morimoto, R.I. (1998). Regulation of the heat shock transcriptional response: cross talk between a family of heat shock factors, molecular chaperones, and negative regulators. *Genes and Development* 12:3788–3796.

Mundt, J.O. (1986) Lactic acid streptococci. In: Bergey's Manual of Systematic Bacteriology, vol 2. ed. Sneath PHA *et al*.pp. 1065–1066. Baltimore, MD: The Williams and Wilkins Co.

Mundt, J.O. and Hammer, J.L. (1968). Lactobacilli on plants. *Applied Microbiology* 16: 1326-1330.

Narberhaus, F. (2002). Alpha-crystallin-type heat shock proteins: socializing minichaperones in the context of a multichaperone network. *Microbiology and Molecular Biology Review*66:64-93.

Nauta, A., van den Burg, B., Karsens, H., Venema, G. and Kok, J.(l997). Design of thermolabile bacteriophage repressor mutants by comparative molecular modeling. *Nature Biotechnology*15:980-983.

Nauta, A., van Sinderen, D., Karsens, H., Smit, E., Venema, G. and Kok, J. (1996). Inducible gene expression mediated by a repressor-operatorsystem isolated from *Lactococcus lactis* bacteriophage rlt. *Microbiology* 19:1331-l 341.

Neu, T. and Henrich, B. (2003). New thermosensitive delivery vector and its use to enable nisin-controlled gene expression in *Lactobacillus gasseri*. *Applied and Environmental Microbiology* 69: 1377–1382.

Noreen, N., Hooi, W.Y., Baradaran, A., Rosfarizan, M., Sieo, C.C., Rosli, M.I., Yusoff, K. and Raha, A.R. (2011) *Lactococcus lactis* M4, a potential host for the expression of heterologous proteins.*Microbial Cell Factory* 10: 28.

Nouaille, S., Ribeiro, L.A., Miyoshi, A., *et al*. (2003). Heterologous protein production and delivery systems for *Lactococcus lactis*. *Genetics and MolecularResearch* 2: 102-11.

Nilsson, D. and Johansen, E. (1994)A conserved sequence in tRNA an rRNA promoters of *Lactococcus lactis*. *Biochimica et Biophysica Acta*1219:139-144.

O'Sullivan, D.J. and Klaenhammer,T.R. (1993). High and low-copy number *Lactococcus* shuttle cloning vectors with features for clone screening. *Gene* 137: 227-231.

Osborn, M., Bron, S., Firth, N., Holsappel, S., Huddleston, A., Kiewiet, R., Meijer, W., Seegers, J., Skurray, R., Terpstra, P., Thomas, C.M., Thorsted, P., Tietze, E.andTurner, S.L. (2000). The evolution of bacterial plasmids. In C. M. Thomas, The Horizontal Gene Pool: Bacterial Plasmids and Gene Spread. Amsterdam, The Netherlands: Harwood Academic Publishers. pp: 301–361.

Outten, C.E. and O'Halloran,T.V. (2001). Femtomolar sensitivity of metalloregulatoryproteins controlling zinc homeostasis. *Science*292**:**2488–2492.

Paek, K.H. and Walker,G.C. (1987). *Escherichia coli* dnaK mutants are inviable at high temperatures. *Journal of Bacteriology* 169:283–290.

Pavan, S., Hols, P., Delcour, J., Geoffroy, M.C., Grangette, C., Kleerebezem, M. and

Mercenier, A. (2000). Adaptation of the nisin-controlled expression system in *Lactobacillus plantarum*: a tool to study in vivo biological effects. *Applied and Environmental Microbiology* 66:4427‑4432.

Pavlova, S.L., Kilic,A.O., Topisirovic, L., Miladinov, N., Hatzos, C., Tao, L. (2002). Characterisation of a cryptic plasmid from *Lactobacillus fermentum* KC5b and its use for constructing a stable *Lactobacillus* cloning vector. *Plasmid* 47: 182-192.

Petrova, P., Miteva, V., Ruiz-Maso, J.A. and del Solar, G. (2003). Structural and functional analysis of pt38, a 2.9kb plasmid of *Streptococcus thermophilus* yogurt strain. *Plasmid* 50: 176-189.

Platteeuw, C., Simons, G. and de Vos,W.M.**(**1993). Use of the *Escherichia coli* β-glucuronidase (*gusA*) gene as a reporter gene for analyzing promoters inlactic acid bacteria. *Applied and Environmental Microbiology*60**:**587–593.

Platteeuw, C., van Alen-Boerrigter, I., van Schalkwijk, S., de Vos, W.M. (1996). Food-grade cloning and expression system for *Lactococcus lactis*. *Applied and Environmental Microbiology* 62:1008–1013.

Posno, M., Leer, R.J., van Luijk, N., van Giezen, M.J.F., Heuvelmans, P.T.H.M., Lokman, B.C. and Pouwels,P.H. (1991). Incompatibility of *Lactobacillus* vectors with replicons derived from small cryptic *Lactobacillus* plasmids and segregational instability of the introduced vectors. *Applied and Environmental Microbiology* 57: 1822-1828.

Pouwels, P.H., Leer, R.J. (1993). Genetics of Lactobacilli, Plasmids and gene expression. *Antonie van Leeuwenhoek* 64:85-107.

Pushnova, E.A., Geier, M., Zhu, Y.S. (2000). An easy and accurate agarose gel assay for quantitation of bacterial plasmid copy numbers. *Analytical Biochemistry* 284: 70–76.

Raha, A.R., Hooi, W.Y., Mariana, N.S., Radu, S., Varma, N.R. and Yusoff, K. (2006).DNA sequence analysis of a small cryptic plasmid from *Lactococcus lactis*subsp. *lactis* M14. *Plasmid* 56: 53–61.

Rasmussen, R.(2001). In: Meuer, S., Wittwer, C., Nakagawara, K. (Eds.), Quantification on the LightCycler. Springer-Verlag, Berlin, Germany pp. 21–34.

Raya, R.R., Fremaux, C. De Antoni, G.L. and Klaenhammer,T.R. (1992). Site-specific integration of the temperate bacteriophage φadhinto the *Lactobacillus gasseri* chromosome and molecularcharacterization of the phage (attP) and bacterial (attB) attachment sites. *Journal of Bacteriology* 174: 5584-5592.

Ringus, D.L., Ivy, R.A., Wiedmann, M. and Boor, K.J. (2012). Salt stress-induced transcription of σB- and CtsR-regulated genes in persistent and non

persistent *Listeria monocytogenes* strains from food processing plants. *Foodborne Pathogen and Desease* 9:198-206

Roberts, R.C., Toochinda, C., Avedissian, M., Baldini, R.L., Gomes, S.L. and Shapiro,L. (1996). Identification of a *Caulobacter crescentus* operon encoding hrcA, involved in negatively regulating heat inducible transcription, and the chaperone gene grpE. *Journal of Bacteriology* 178: 1829–1841

Roche, (2001). LightCycler: Relative Quantification. Technical Note, LC13/2001, pp. 1–28.

Rigoulay, C., Poquet, I., Madsen, S.M. and Gruss, A. (2004). Expression of the *Staphylococcus aureus* surface proteins Htra(1) and Htra(2)in *Lactococcus lactis.FEMS Microbiology Letters* 237:279–288.

Rossi, F., Rudella, A., Marzotto, M. and Dellaglio, F. (2001) Vector-free cloning of abacterial endo-1,4-β-glucanase in Lactobacillusplantarum and its effect on the acidifying activity in silage: useof a recombinant cellulolytic *Lactobacillus plantarum* as silageinoculant. *Antonie van Leeuwenhoek* 80:139–147.

Sambrook, J. and Russell, D.W. (2001). *Molecular cloning:* a laboratory manual, third edition. New York: Cold Spring Harbor Laboratory Press.

Sambrook, J. Fritsch, E.F. and Maniatis, T. (1989). *Molecular cloning:* a laboratory manual, second edition. Cold Spring Harbor Laboratory Press.

Sanchez, A.-H., Rejano, L., Montan˜o, A. and de Castro,A. (2001). Utilization at high pH of starter cultures of lactobacilli for Spanish-style green olive fermentation. *International Journal of Food Microbiology*. 67:115–122.

Sanchez, C., Neves, A.R., Cavalheiro, J., dos Santos, M.M., Garcia-Quintans, N., Lopez, P. and Santos, H. (2008) Contribution of citrate metabolism to the growth of Lactococcus lactis CRL264 at low pH. *Applied of Environmental Microbiology*. 74: 1136–1144.

Sano, K., Otani, M., Okada,Y., Kawamura, R.,Umesaki, M., Ohi, Y., Umezawa, C. and Kanatani,K. (1997). Identification of the replication region of the *Lactobacillus acidophilus* plasmid pLA106. *Gene*186: 255-262.

Savijoki, K., Kahala, M. and Palva, A. (1997). High level heterologous protein production in *Lactococcus* and *Lactobacillus* using a new secretion system based on the *Lactobacillus brevis* S-layer signals. *Gene* 186:255–262

Schiffrin, E.J. and Blum, S. (2001). Food processing: probiotic microorganisms for beneficial foods. *Current Opinion in Biotechnology* 12: 499–502.

Schirmer, E.C., Glover, J.R. Singer, M.A. and Lindquist,S. (1996). HSP100/Clp proteins: a common mechanism explains diverse functions. *Trends in Biochemical Sciences* 21:289–296.

Schulz, A., Tzschaschel, B. and Schumann,W. (1995). Isolation and analysis of mutants of the dnaK operon of *Bacillus subtilis*. Molecular Microbiology 15:421–429.

Seegers, J.F., Bron, S., Franke, C.M., Venema, G. and Kiewiet, R. (1994). The majority of lactococcal plasmids carry a highly related replicon. *Microbiology*. 140: 1291–1300.

Segal, R. and Ron, E.Z. (1993). Heat shock transcription of the groESL operon of *Agrobacterium tumefaciens* may involve a hairpin-loop structure. *Journal of Bacteriology* 175: 3083–3088.

Segal, R. andRon,E.Z. (1996). Regulation and organization of the groE and dnaK operons in Eubacteria. *FEMS Microbiology Letters* 138: 1–10.

Shareck, J., Choi, Y., Lee, B. and Miguez, C.B. (2004). Cloning vectors based on crytic plasmids isolated from lactic acid bacteria: their characteristics and potential application in biotechnology. *Critical Review in Biotechnology* 24:155-208.

Simon, D. and Chopin,A. (1988). Construction of a vector family and its use for molecular cloning in *Streptococcus lactis*. *Biochimie*70: 559-566.

Solow, B.T. and Somkuti, G.A. (2000). Comparison of low-molecular-weight heat stress proteins encoded on plasmids in different strains of *Streptococcus thermophilus*.*Current Microbiology* 41: 177-181.

Somkuti, G.A., Solaiman, D.K. and Steinberg, D.H. (1998). Structural and functional properties of the hsp l6.4-bearing plasmid pER341 in *Streptococcus thermophilus*.*Plasmid* 40: 61-72.

Somkuti, G.A. and Steinberg, D.H. (1999). Distribution of plasmid-borne stress protein genes in Streptococcus thermophilus and other lactic acid bacteria.*Current Microbiology* 38: 43-7.

Sørvig, E., Gronqvist, S., Naterstad, K., Mathiesen, G., Eijsink, V.G. and Axelsson, L. (2003). Construction of vectors for inducible gene expression in *Lactobacillus sakei* and *L. plantarum*. *FEMS Microbiology Letters* 229, 119–126.

Sørvig, E., Mathiesen, G., Naterstad, K., Eijsink, V. G. and Axelsson, L. (2005). High-level, inducible gene expression in *Lactobacillus sakei*and *Lactobacillus plantarum* using versatile expression vectors. *Microbiology* 151: 2439–2449.

Spath, K., Heinl, S. and Grabherr, R. (2012).“Direct cloning in *Lactobacillus plantarum*: Electroporation with non-methylated plasmid DNA enhances transformation efficiency and makes shuttle vectors obsolete”. *Microbial Cell Factory* 11:141.

Stanley, E., L. Walsh, A. van der Zwet, G. F. Fitzgerald, and D. van Sinderen. (2000). Identification of four loci isolated from two *Streptococcus thermophilus*phage genomes responsible for mediating bacteriophage resistance. *FEMS Microbiology Letters*182**:**271–277.

Stiles, M.E., and Holzapfel, W. H. (1997). Lactic acid bacteria of foods and their current taxonomy. *InternationalJournal of Food Microbiology* 36: 1–29.

Stortz, G. and Hengge-Aronis, R. (2000). *Bacterial Stress Responses*(p 485). ASM Press, Washington, DC.

Tannock, G.W., Luchansky, J.B.,Miller, L., Connell, H., Thode- Andersen, S., Mercer, A.A. and Klaenhammer,T.R. (1994). Molecular characterization of a plasmid-borne (pGT633) erythromycin resistancedeterminant (ermGT) from *Lactobacillus reuteri* 100-63. *Plasmid*31: 60-71.

Terzaghi, B.E. and Sandine, W.E. (1975) Improved medium for lactic streptococci and their bacteriophages. *Journal of Applied Microbiology* 29: 807-813.

Teusink, B., Bachmann, H. andMolenaar, D. (2011) Systems biology of lactic acid bacteria: a critical review. *Microbial Cell Factory* 10: 11.

Thomas, J.G. and Baneyx, F. (2000). ClpB and HtpG facilitate de novo protein folding in stressed *Escherichia coli* cells. *Molecular Microbiology* 36:1360 1370.

Tomoyasu, T., Gamer, J., Bukau, B., Kanemori, M.,Mori, H., Rutman, A.J., Oppenheim, A.B. and Yura, T. (1995). *Escherichia coli* FtsH is a membrane bound, ATP-dependent protease which degrades the heat-shock transcription factor sigma 32. *EMBO Journal*14: 2551–2560

Trias, R., Bañeras, L., Montesinos, E. and Badosa,E. (2008). Lactic acid bacteria from fresh fruit and vegetables as biocontrol agents of phytopathogenic bacteria and fungi. International Microbiology 11: 231–236.

vanBogelen, R.A., Greis, K.D., Blumenthal, M., Tani, T.H. and Matthews, R. (1999). Mapping regulatory networks in microbial cells. *Trends in Microbiology* 7: 320–328.

van de Guchte, M., Penaud, S., Grimaldi, C., Barbe, V., Bryson, K., Nicolas, P., Robert, C., Oztas, S., Mangenot, S., Couloux, A., Loux, V., Dervyn, R., Bossy, R., Bolotin, A., Batto, J.M., Walunas, T., Gibrat, J.F., Bessieres, P., Weissenbach, J., Ehrlich, S.D., and Maguin E. (2006). The complete genome

sequence of *Lactobacillusbulgaricus* reveals extensive and ongoing reductive evolution. Proc. Natl. Acad. Sci. USA 103: 9274‑9279.

van der Vossen, J.M.B.M., Kok, J. and Venema, G. (1985). Construction of cloning, promoter-screening, and terminator-screening shuttle vectors for *Bacillus subtilis* and *Streptococcus lactis*. *Applied and Environmental Microbiology* 50: 540-542.

van Niel, E.W.J. and Hahn-Hägerdal, B. (1999).Nutrients requirements of lactococci in defined growth media. *Applied Microbiology and Biotechnology*. 52;617 627.

van Sinderen, D., Karsens, H., Kok, J., Terpstra, P., Ruiters, M.H.J.,Venema, G.and Nauta, A. (1996). Sequence analysis and molecular characterization of the temperate lactococci bacteriophage r1t. *Molecular Microbiology* 19:1343-l 355.

Varmanen, P., lngmer, H. and Vogensen, F.K. (2000).ctsR of Lactococcus lactis encodes a negative regulator of clp gene expression. *Microbiology* 146: 1447-1455.

Vigh, L., Maresca, B. and Harwood,J.L. (1998). Does the membrane's physical state control the expression of heat shock and other genes? *Trends in Biochemical Sciences* 23:369–374.

von Wright, A., Wessels, S., Tynkkynen, S. and Saarela, M. (1990). Isolation of a replication region of a large lactococcal plasmid and use in cloning of a nisin resistance determinant. *Applied andEnvironmental Microbiology* 56: 2029–2035.

Vujcic, M. and Topisirovic,L..(1993). Molecular analysis of the rollingcircle replicating plasmid pA1 of *Lactobacillus plantarum* A112. Applied and Environmental Microbiology 59: 274-280.

Walker, D.C. and Klaenhammer,T.R. (1994). Isolation of a novel IS3 group insertion element and construction of an integration vector for *Lactobacillus* spp. Journal of Bacteriology 176: 5330-5340.

Waterfield, N.R., LePage, R.W.F., Wilson, P.W. and Wells, J.M. (1995). The isolation of lactococcal promoters and their use in investigating bacterial luciferase synthesis in *Lactococcus lactis*. *Gene* 165:9-l 5.

Wegmann, U., O'Connell-Motherway, M., Zomer, A., *et al*. (2007). Complete genome sequence of the prototype lactic acid bacterium *Lactococcuslactis* subsp. *cremoris* MG1363. Journal of Bacteriology 189: 3256-3270.

Weisburg, W., Barns, S., Pelletier, D., & Lane, D. (1991). 16S ribosomal DNA amplification for phylogenetic study. *Journal of Bacteriology*173: 697-703.

Wessels, S., Axelsson, L., Hansen, E.B., De Vuyst, L., Laulund, S., Lähteenmäki, L, Lindgren, S., Mollet, B., Salminen, S. and von Wright, A. (2004) The lactic acid bacteria, the food chain, and their regulation. *Trends in Food Science and Technology* 15, 498–505.

West Des Moines, I.A., Wegmann, U., O'Connell-Motherway, M., Zomer, A., *et al*. (2007). Complete genome sequence of the prototype lactic acid bacterium *Lactococcus lactis* subsp. *cremoris* MG1363. *Journal of Bacteriology* 189: 3256-3270.

Wilhelm, J., Pingoud, A., Hahn, M., (2003). Real-time PCR-based method for the estimation of genome sizes. *Nucleic Acids Research* 31: e56.

Whelan, J.A., Russel, N.B., Whelan, M.A. (2003). A method for the absolute quantificationof cDNA using real time PCR. *Journal of Immunological Methods* 278: 261–269.

Wood, B.J. and Warner, P. J. (2003). *Genetics of Lactic Acid Bacteria* (Vol. 3). New York: Kluwer Academic/ Plenum Publisers.

Yamamoto, N., and Takano, T. (1996). Isolation and characterisation of a plasmid from*Lactobacillus helveticus* CP53. *Bioscience Biotechnology and Biochemistry* 60: 2069-2070.

Yura, T., Nagai, H. and Mori, H. (1993). Regulation of the heat-shock response in bacteria. *Annual Review of Microbiology*47: 321-350.

Yura, T., Tobe, T., Ito, K. and Osawa,T. (1984). Heat shock regulatory gene (htpR) of*Escherichia coli* is required for growth at high temperature but is dispensable at lowtemperature. *Proceedings of the National Academy of Sciences USA*81: 6803–6807.

Zhou, X.X., Li, W.F., Ma, G.X. and Pan, Y.J. (2006). The nisin-controlled gene expression system: construction, application and improvements. *Biotechnology Advances* 24: 285-295.

Zhou, Y.N., Kusukawa, N., Erickson, J.W., Gross, C.A. and Yura,T. (1988). Isolation and characterization of *Escherichia coli* mutants that lack the heat shock sigma factor sigmasuper. *Journal of Bacteriology* 170: 3640–3649

Zhu, Y., Zhang, Y.P. and Li, Y. (2009). Understanding the industrial application potential of lactic acid bacteria through genomics. *Applied Microbiology and Biotechnology* 83, 597−610.