UNIVERSITI PUTRA MALAYSIA

NISP AS A POTENTIAL SURFACE ANCHOR FOR PANDEMIC H1N1 2009 HAEMAGGLUTININ (HA1) VIRAL EPITOPE IN Lactococcus lactis

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FBSB 2014 39

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By

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

December 2014

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

NISP AS A POTENTIAL SURFACE ANCHOR FOR PANDEMIC H1N1 2009 HAEMAGGLUTININ (HA1) VIRAL EPITOPE IN _Lactococcus lactis_

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Chairman: Professor Raha Abdul Rahim, PhD
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The rapid worldwide spread of the pandemic H1N1 2009 influenza A virus in the early 2009 has contributed to high morbidity and mortality especially in children, the elderly and immuno-compromised individuals. Current available influenza vaccines are effective in terms of providing immunity protection but there are several limitations and disadvantages such as allergic reactions, biosafety issues and limited production capacity. This study uses *Lactococcus lactis* as a vehicle for cloning of the haemagglutinin (HA1) epitope of the pandemic H1N1 2009 influenza virus. The HA1 epitope was amplified and cloned into a *L. lactis* vector together with the usp45 signal peptide and nisP anchor protein (nisP-anc) in order to assemble a surface displayed HA1 recombinant *L. lactis*. The recombinant *L. lactis* were able to express HA1 epitope protein when induced with nisin and was further confirmed with Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) analysis. The recombinant host cells were further examined by using whole cell ELISA, fluorescence microscopy and flow cytometry to detect the surface displayed HA1 epitope protein on their surfaces. Despite it could not be confirmed that HA1 epitope has been successfully surface displayed, the recombinant cells were fed to BALB/c mice host to observe for its capability to induce immunogenic response. From the analysis of antigen-specific serum antibody by sandwich ELISA, significant anti-HA1 sIgA antibody was produced in the mice fecal suspension of mice group NZ9000 (pNZ:HN) when compared to the negative control NZ9000 (pNZ8048) suggesting that high specific anti-HA1 sIgA antibody was produced in the mice rectum. This study was conducted to gain more insights of the potential of *L. lactis* as a vaccine vehicle and future optimization can be adopted to further enhance its capability in vaccine delivery.

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

NISP SEBAGAI POTENSI PAPARAN PERMUKAAN EPITOP VIRUS PANDEMIK H1N1 2009 HEMAGLUTININ PADA REKOMBINAN _Lactococcus lactis_

Oleh

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Virus influenza A Pandemik H1N1 2009 yang muncul pada awal tahun 2009 telah merebak dengan cepat di seluruh dunia dan mengakibatkan morbiditi dan mortaliti terutama sekali di kalangan kanak-kanak, warga emas dan individu yang mempunyai sistem imun yang lemah. Vaksin influenza yang kini didapati adalah efektif dari segi perlindungan keimunan tetapi terdapat beberapa batasan dan keburukan seperti reaksi alergi, isu-isu keselamatan biologi dan keupayaan pengeluaran yang terhad. Kajian ini menggunakan *Lactococcus lactis* sebagai pembawa untuk pengklonan epitop hemaglutinin (HA1) dari virus influenza A Pandemik H1N1 2009. Epitop HA1 telah diamplifikasikan dan diklonkan ke dalam vektor *L. lactis* bersama dengan peptida isyarat usp45 dan protein permukaan bersauh nisP (nisP-anc) untuk membentuk rekombinan *L. lactis* yang mempamerkan epitop HA1 dipermukaannya. Rekombinan *L. lactis* ini berkeupayaan untuk mengekspres protein epitop HA1 yang diaruh oleh nisin dan seterunya disahkan dengan menggunakan analisa "Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS)". Sel-sel rekombinan ini seterusnya disahkan dengan menggunakan ELISA seluruh sel, mikroskopi fluoresen, dan "flow cytometry" untuk mengesan epitop HA1 yang dipamerkan di permukaan hos sel *L. lactis*. Walaupun tiada pengesahan yang epitop HA1 telah dengan jayanya dipaparkan di permukaan *L. lactis*, sel-sel rekombinan ini telah diuji dalam hos tikus BALB/c untuk mengesan keupayaannya untuk menghasilkan gerak balas imunogenik. Daripada analisa serum antibodi spesifik-antigen dengan menggunakan ELISA sandwic, antibodi sIgA anti-HA1 yang tinggi dalam suspensi tinja tikus dapat dikesan dalam kumpulan tikus NZ9000 (pNZ:HN) berbanding dengan kumpulan tikus kawalan negatif NZ9000 (pNZ8048) mencadangkan bahawa antibodi sIgA anti-HA1 yang tinggi telah dihasilkan di dubur tikus-tikus tersebut. Kajian ini dijalankan untuk mendapat pengetahuan dan menentukan potensi *L. lactis* sebagai pembawa vaksin dan pengoptimuman di masa akan datang seterusnya berupaya meninggikan keupayaannya dalam penyampaian vaksin.

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to my supervisor, Prof. Dr. Raha Abdul Rahim for her endless support, advice and guidance throughout my entire studies. Although her time is very limited, she always try to make herself available to her students, patiently listen and guide her students whole heartedly making sure they are on the right path and most importantly, never give up on them. My heartfelt thanks to Prof. Dr. Khatijah Yusoff, Dr. Chang Li Yen and Prof. Dr. Sazaly Abu Bakar, for their extended knowledge and support which inspires me in my studies.

To Dr. Adelene Song and my beloved labmates, I will always cherish the wonderful experience and journey together that we had. We have been through thick and thin together and I wish all the best to you and your future. To Jocelyn Jee, thank you for your help and company. It was a great pleasure working together with you and I hope our path will cross again in the future.

To my parents, I thank you for your support; morally and financially, you are always there for me and encouraging me to always reach for greater heights. I love you both very much.

Lastly, I would like to thank Universiti Putra Malaysia for awarding me with the Graduate Research Fellowship.

I certify that a Thesis Examination Committee has met on 1 December 2014 to conduct the final examination of Stella Siaw Xiu Joan on her thesis entitled "*NISP* as a Potential Surface Anchor for Pandemic H1N1 2009 Haemagglutinin (HA1) Viral Epitope in *Lactococcus lactis*" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A)106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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LIST OF ABBREVIATIONS

A260	-	absorbance at 260 nm
A450	-	absorbance at 450 nm
aa	-	amino acid
AcmA	-	N-acetylmuraminidase
BLAST	-	basic local alignment search tool
Bp	-	base pair
BSA	-	bovine serum albumin
$CaCl_2$	-	calcium chloride
cDNA	-	complimentary DNA
cfu	-	colony forming unit
CO_2	-	carbon dioxide
C-terminal	-	the carboxyl-terminal (-COOH) of a polypeptide
dH_2O	-	distilled water
DNA	-	deoxyribonucleic acid
dNTPs	-	deoxyribonucleotide triphosphate
EDTA	-	ethylenediamine tetraacetate
ELISA	-	enzyme-linked immunosorbant assay
EtBr	-	ethidium bromide
GMO	-	genetically modified organisms
GRAS	-	generally regarded as safe
H	-	hour
HA1	-	haemagglutinin 1
HCl	-	hydrochloric acid
His	-	histidine
HRP	-	horseradish peroxide
i.p	-	intraperitoneally
i.v	-	intravenously
IPTG	-	isopropyl-D-galactopyranoside
kb	-	kilo base pair
kDa	-	kilo Daltons
LAB	-	lactic acid bacteria
LB	-	Luria Bertani
M	-	molarity
MCS	-	multiple cloning site
$MgCl_2$	-	magnesium chloride
min	-	minute
ml	-	milliliter
mM	-	milliMolar
mRNA	-	messenger RNA
NaCl	-	sodium chloride
NAG	-	N-acetylglucosamine
NAM	-	N-acetylmuramic acid
ng	-	nanogram
Ni^{2+}	-	nickel sulphate ion
NisP	-	serine protease
nisP-anc	-	NisP anchor protein
N-terminal	-	the amino-terminal (NH2) of a polypeptide
^oC	-	degrees centigrade

OD	-	optical density
ORF	-	open reading frame
PBS	-	phosphate buffered saline
PCR	-	polymerase chain reaction
PEG	-	polyethylene glycol
Pfu	-	*pyrococcus furiosus*
pH	-	isoelectric point
POD	-	peroxidase
PVDF	-	immobilon-polyvinydene difluoride
RBS	-	ribosomal binding site
RE	-	restriction enzyme
RNA	-	ribonucleic acid
RNase	-	ribonuclease
rpm	-	revolution per minute
rRNA	-	ribosomal RNA
RT	-	room temperature
s	-	second
SDS-PAGE	-	sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SOE	-	Splicing by overlap extension
sP.	-	species
subsp.	-	subspecies
TAE	-	tris-acetate-EDTA
Taq	-	Thermus aquaticus
TCA	-	tri-carboxylic acid
TE	-	tris EDTA
TEMED	-	tetramethyl-ethylene diamine
TGS	-	tris-glycine-SDS
TMB	-	3,3',5,5'-Tetramethylbenzidine
TTFC	-	tetanus toxin fragment C
U	-	unit
UV	-	ultraviolet
v/v	-	volume per volume
w/v	-	weight per volume
%	-	percent/percentage
µl	-	microliter
µg	-	microgram

CHAPTER 1

INTRODUCTION

1.1 Introduction

Pandemic H1N1 2009 influenza A virus which emerged in early 2009 has spread rapidly across the globe and contributed to morbidity and mortality especially in children, elderly and immune-compromised individuals. Although antiviral agents are able to treat influenza infections, vaccination is still the best method for controlling influenza. Current available influenza vaccines are live attenuated viruses that are propagated in embryonated chicken eggs. It is effective in terms of providing immunogenic protection but there are several limitations and disadvantages of this method such as allergic reactions to the egg protein, biosafety issues and limited production capacity.

The use of *Lactococcus lactis*, a lactic acid bacteria (LAB), in the production of recombinant vaccine is still less exploited compared to attenuated pathogens such as Salmonella (Levine *et al.*, 1996; Branger *et al.*, 2007). Their generally recognized as safe status and the availability of genetic tools for recombinant gene expression make them attractive to be used as potential vaccine vehicles. It can also overcome the disadvantages of egg-based vaccines and reversions. Several antigen specific immune responses have been successfully obtained using *L. lactis* as the vaccine carrier (Mozzi *et al.*, 2010). This study is a novel step towards the use of nisP-anc and LAB, *L. lactis* as a vehicle for the production of pandemic H1N1 2009 influenza virus vaccine.

1.2 Hypotheses

The hypotheses for this study are:

1. The NisP anchor protein (nisP-anc) will be able to surface display the HA1 pandemic H1N1 2009 viral epitope on *L. lactis* host cells.
2. The surface displayed recombinant *L. lactis* harbouring the HA1 viral epitope will stimulate both the cell-mediated and humoral immune responses in the BALB/c mouse model.

1.3 Problem statements

1. The production of current licensed pandemic H1N1 2009 vaccine cannot be scaled up easily.
2. Viruses propagated in the chicken eggs may be may be slightly modified during incubation period.
3. Inconvenient vaccination procedure such as intramuscular injection.
4. Recombinant vaccines produced with pathogenic microbes may pose threat to vaccinated person's health.

1.4 Objectives

The main objectives of this work include:

1. To construct an expression vector for *L. lactis* constituting of HA1 pandemic H1N1 2009 viral epitope together with usp45 signal peptide and nisP-anc.
2. To optimize expression conditions of the recombinant *L. lactis* constructs to improve gene expression.
3. To analyse surface display of the HA1 viral epitope on the *L. lactis* cell surface.
4. To investigate the immunogenic effect of recombinant *L. lactis* harbouring HA1 viral epitope when fed orally to BALB/c mice host.

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