



UNIVERSITI PUTRA MALAYSIA

**BACTERIOPHAGE AS A POTENTIAL THERAPEUTIC AGENT
AGAINST SALMONELLA ENTERICA SEROVAR TYPHIMURIUM IN
LOCAL BROILER CHICKEN**

WONG CHUAN LOO

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BROILER CHICKEN**

By

WONG CHUAN LOO

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June 2011

Chairperson: Associate Professor Sieo Chin Chin, PhD

Faculty: Faculty of Biotechnology and Biomolecular Sciences

Global expansion of the livestock industry and demand for the production of livestock products have increased noticeably in many developing countries. In Malaysia, poultry industry represents by far the largest proportion in the livestock industry in terms of livestock output-value. The marked expansion of poultry's products in order to meet the demand for poultry meat led to the rapid increase in the incidence of zoonotic diseases and emergence of multi-drug resistant microorganisms, primarily resulted from the application of growth-promoting drugs in the animal production line. Salmonellosis remains one of the most frequently reported zoonotic diseases with *Salmonella enterica* serovar Typhimurium being the pre-dominant implicated serotype that is resistant to a great variety of antibiotics. In recent years, *Salmonella* specific-bacteriophages have been reported as a plausible alternative to antibiotics. Thus, the present study was carried out with the aim to isolate bacteriophages against local *S. Typhimurium*. The bacteriophage isolates were analysed by electron microscopy and their genomes were isolated to study their size and type, and restriction enzyme profile patterns. The structural proteins of the

phages were also studied by SDS-PAGE. Phage growth was characterised by the one-step kinetics growth curve, adsorption rate, multiplicity of infection (MOI) ratios and the effects of different pHs and temperatures on phage-host interaction. Subsequently, the efficacy of a selected phage to reduce the host bacteria load *in vivo* was evaluated.

In the present study, three lytic bacteriophages namely Φ_{st1} , Φ_{st5} and Φ_{st10} were isolated from poultry faecal materials. The morphology of these phages indicated that they belong to the *Siphoviridae* family. Φ_{st1} was found to demonstrate a broader host range in which it was able to infect *S. enterica* serovar Hadar (*S. Hadar*) apart from *S. Typhimurium*, the original host in which it was isolated from. All the three phages were DNA phages harbouring a genome about 125 kbp size. Although all the three phages revealed similar protein profile when analysed using SDS-PAGE, they can be differentiated with distinctive restriction digestion profiles of *EcoRI* and *BamHI*. All three phages have a latent period ranging from 40-50 min and generated a burst size of 22 (Φ_{st1}), 10 (Φ_{st5}) and 29 (Φ_{st10}) particles per infective center, respectively. MOI optimisation results revealed that MOI ratio of 0.1 generated the highest bacteria reduction rate (~ 6 log cfu/ml) and an increment of ~ 4 log pfu/ml in phage titer. Φ_{st1} was found to produce the highest adsorption rate (86.1%) within the first 5 min of infection, and subsequently followed by Φ_{st10} (82.1%) and Φ_{st5} (60.4%). Among the three phage isolates, Φ_{st5} demonstrated a broader spectrum of pH tolerance (pH 5-11), in which ≤ 0.1 log pfu/ml decrease in phage titer was detected. However, under different pH conditions, Φ_{st1} was observed to demonstrate the highest adsorption rates (88.4-92.2%) at pH 7-11, whereby the highest bacteria reduction (6.6 log cfu/ml) was observed at pH 9. In general, all the three phage

isolates were stable at the temperatures (25 °C, 37 °C, 42 °C and 60 °C) tested with less than 0.5 log pfu/ml decrease in phage titer after 3 h of incubation. The three phages demonstrated the highest (5.9-6.4 log cfu/ml) and insignificant different reduction rate of host cells at 37 °C and 42 °C. Based on the overall characteristics of the phage isolates, $\Phi st1$ was selected for subsequent *in vivo* study. With the high titer application of $\Phi st1$ ($\sim 10^{12}$ pfu/ml) in chicks challenged with $\sim 10^{10}$ cfu/ml of *S. Typhimurium*, $\Phi st1$ was found to reduce the *S. Typhimurium* count by 5.5 log cfu/ml within 6 h of post-challenge. Systemic infection was also reduced in this group of chickens. Unlike the untreated chickens, *S. Typhimurium* was not detected in the liver, heart and spleen of chicks throughout the sampling period in $\Phi st1$ treated chickens. $\Phi st1$ (~ 1.6 log pfu/ml) was found to persist in the caeca wall of chicks at and after 48 h post-challenge.

In conclusion, $\Phi st1$ might serve as a potential therapeutic agent to control and reduce the *Salmonella* count in caeca content of chickens. However, further optimisation of both dosage and alternative routes of phage administration should be carried out to enhance the efficacy of the phage.

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

**POTENSI BAKTERIOFAJ SEBAGAI AGEN TERAPEUTIK UNTUK
SALMONELLA ENTERICA SEROVAR TYPHIMURIUM YANG TERSEBAR
LUAS DI KALANGAN AYAM PEDAGING TEMPATAN**

Oleh

WONG CHUAN LOO

Jun 2011

Pengerusi: Profesor Madya Sieo Chin Chin, PhD

Fakulti: Fakulti Bioteknologi dan Sains Biomolekul

Perluasan global dalam industri ternakan dan peningkatan permintaan untuk pengeluaran produk ternakan semakin ketara di negara-negara membangun. Industri peternakan ayam di Malaysia merupakan salah satu industri ternakan yang terbesar dari segi nilai keluaran-ternakan. Dalam usaha memenuhi permintaan daging ayam yang tinggi, insiden penyakit zoonosis dan mikroorganisma yang mempunyai daya ketahanan antibiotik juga meningkat secara drastik akibat daripada penggunaan berleluasa ‘ubat peningkatan pertumbuhan’. Salmonellosis merupakan salah satu daripada penyakit zoonosis yang sering dilaporkan, di mana *Salmonella enterica* serovar Typhimurium merupakan serotaip *Salmonella* yang dominan dan rintang terhadap pelbagai antibiotik. Dalam beberapa tahun ini, bakteriofaj yang khusus terhadap *Salmonella* telah dilaporkan sebagai alternatif terhadap antibiotik. Dengan demikian, kajian ini bertujuan untuk pemencilan bakteriofaj *S. Typhimurium*. Isolat bakteriofaj dianalisis dengan menggunakan mikroskop elektron dan genom bakteriofaj dipencilkan untuk kajian jenis dan saiz, dan juga analisis profil genom dengan menggunakan enzim pembatas. Struktur protein faj juga dianalisis dengan

SDS-PAGE. Ciri-ciri pertumbuhan bakteriofaj misalnya ‘kinetik keluk pertumbuhan selangkah’, ‘kadar penjerapan’, nisbah MOI dan pengaruh pH serta suhu yang berbeza pada interaksi di antara bakteria dan bakteriofaj dikaji. Selanjutnya, keberkesanan bakteriofaj dalam mengurangkan jumlah bakteria perumah dinilai secara *in vivo*.

Dalam kajian ini, tiga bakteriofaj litik iaitu Φ *st1*, Φ *st5* dan Φ *st10* telah dipencilkan daripada bahan kumuh ternakan poulti. Morfologi keseluruhan ketiga-tiga bakteriofaj ini menunjukkan bahawa faj ini berasal dari famili *Siphoviridae*. Φ *st1* didapati menunjukkan julat perumah yang lebih luas di mana ia mampu menjangkiti *S. enterica* serovar Hadar (*S.Hadar*) selain dari *S. Typhimurium*, perumah asal di mana ia dipencil. Ketiga-tiga bakteriofaj tersebut adalah bakteriofaj DNA dengan saiz genom sekitar 125 kbp. Walaupun ketiga-tiga faj mendedahkan profil protein yang serupa melalui analisis SDS-PAGE, ianya dapat dibezakan dengan profil ‘pencernaan pembatasan’ oleh *EcoRI* dan *BamHI*. Ketiga-tiga faj tersebut mempunyai tempoh pendam antara 40-50 minit dan masing-masing menghasilkan saiz letusan sebanyak 22 (Φ *st1*), 10 (Φ *st5*) dan 29 (Φ *st10*) zarah per pusat jangkitan. Hasil pengoptimuman MOI dari faj menunjukkan bahawa nisbah MOI 0.1 mempunyai kadar pengurangan bakteria tertinggi (~ 6 log cfu/ml) dan peningkatan sebanyak ~4 log pfu/ml dalam titer faj. Φ *st1* didapati menghasilkan kadar penjerapan tertinggi (86.1%) dalam 5 minit pertama jangkitan, dan kemudian diikuti oleh Φ *st10* (82.1%) dan Φ *st5* (60.4%). Di antara tiga isolat faj, Φ *st5* menunjukkan spektrum toleransi pH (pH 5-11) yang lebih luas, di mana penurunan ≤ 0.1 log pfu/ml dalam titer faj dikesan. Namun, dalam keadaan pH yang berbeza, Φ *st1* didapati menunjukkan kadar penjerapan tertinggi (88.4-92.2%) pada pH 7-11 dan

pengurangan bakteria tertinggi (6.6 log cfu /ml) pada pH 9. Secara umumnya, ketiga-tiga isolat faj adalah stabil pada suhu-suhu yang diuji (25 °C, 37 °C, 42 °C dan 60 °C) dengan kurang daripada 0.5 log pfu/ml penurunan dalam titer faj. Ketiga-tiga faj menunjukkan tahap pengurangan sel perumah yang tertinggi (5.9-6.4 log cfu/ml) pada suhu 37 °C dan 42 °C. Tiada perbezaan yang nyata diperhatikan pada tahap pengurangan bakteria di antara dua suhu ini. Berdasarkan ciri-ciri keseluruhan isolat faj, Φ st1 dipilih untuk kajian *in vivo* yang selanjutnya. Dengan inokulasi titer Φ st1 yang tinggi ($\sim 10^{12}$ pfu/ml) pada anak ayam yang dijangkiti dengan $\sim 10^{10}$ cfu/ml *S. Typhimurium*, Φ st1 didapati mengurangkan bilangan *S. Typhimurium* sebanyak 5.5 log cfu/ml dalam tempoh 6 jam selepas jangkitan. Jangkitan sistemik dalam kumpulan ayam ini juga berkurang. Tidak seperti ayam yang tidak dirawat, *S. Typhimurium* tidak dapat dikesan di dalam hati, jantung dan limpa ayam sepanjang tempoh pengambilan sampel dalam ayam yang dirawat dengan Φ st1. Φ st1 (~ 1.6 log pfu/ml) masih dapat dikesan pada dinding sekum ayam pada dan selepas 48 jam ayam dijangkit.

Sebagai kesimpulan, Φ st1 berpotensi sebagai agen terapeutik untuk mengawal dan mengurangkan jumlah *Salmonella* dalam kandungan sekum ayam. Walaubagaimanapun, pengoptimuman selanjutnya pada dos dan laluan alternatif untuk aplikasi faj seharusnya dijalankan untuk meningkatkan keberkesanan faj tersebut.

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I certify that a Thesis Examination Committee has met on 13 June 2011 to conduct the final examination of Wong Chuan Loo on her thesis entitled “Bacteriophage as a Potential Therapeutic Agent Against *Salmonella enterica* Serovar Typhimurium in Local Broiler Chicken” in accordance with the Universities and University College 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.

Members of the Thesis Examination Committee were as follows:

Wan Zuhainis binti Saad, PhD

Senior Lecturer

Faculty of Biotechnology and Biomolecular Sciences

Universiti Putra Malaysia

(Chairman)

Norazizah binti Shafee, PhD

Associate Professor

Faculty of Biotechnology and Biomolecular Sciences

Universiti Putra Malaysia

(Internal Examiner)

Abdul Rani bin Bahaman, PhD

Professor

Faculty of Veterinary Medicine

Universiti Putra Malaysia

(Internal Examiner)

Sharifah Syed Hassan, PhD

Associate Professor

School of Medicine and Health Sciences

Monash University Sunway Campus

Malaysia

(External Examiner)

NORITAH OMAR, PhD

Associate Professor and Deputy Dean

School of Graduate Studies

Universiti Putra Malaysia

Date: 26 July 2011

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:

Sio Chin Chin, PhD

Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Ho Yin Wan, PhD

Fellow Researcher
Institute of Bioscience
Universiti Putra Malaysia
(Member)

Norhani Abdullah, PhD

Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

Tan Wen Siang, PhD

Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

HASANAH MOHD. GHAZALI, PhD

Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION

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

WONG CHUAN LOO

Date: 13 June 2011

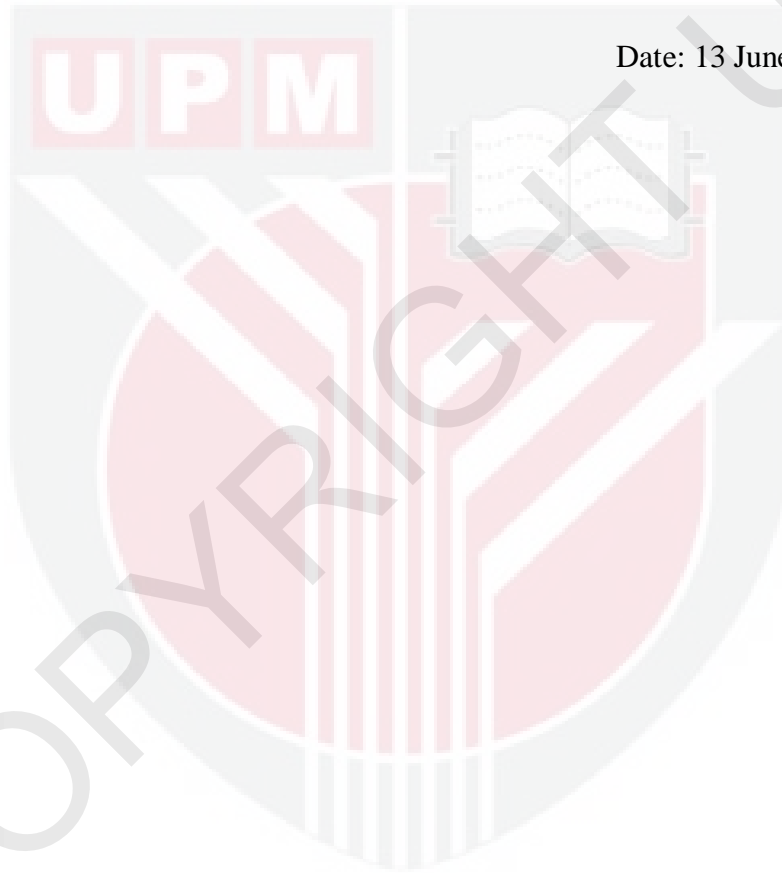


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