



UNIVERSITI PUTRA MALAYSIA

**CHARACTERIZATION OF BACTERIOPHAGES FOR *E. coli* CAUSING
COLIBACILOSIS IN CHICKEN**

NUR ATHIRAH NUR AZAMI

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PENGESAHAN

Dengan ini adalah disahkan bahawa projek yang bertajuk “Characterization of Bacteriophages for *Escherichia coli* Causing Colibacillosis in Chicken” telah disiapkan serta dikemukakan kepada Jabatan Mikrobiologi oleh NUR ATHIRAH BINTI NOOR AZAMI (161050) sebagai syarat untuk kursus BMY 4999 Projek.

Disahkan oleh:

.....

Tarikh:

Prof. Madya Dr. Sieo Chin Chin

Penyelia

Jabatan Mikrobiologi

Fakulti Bioteknologi dan Sains Biomolekul

Universiti Putra Malaysia

.....

Tarikh:

Prof. Madya Dr. Muhajir Hamid

Ketua

Jabatan Mikrobiologi

Fakulti Bioteknologi dan Sains Biomolekul

Universiti Putra Malaysia

ABSTRACT

The emergence of pathogenic bacteria resistant towards most of the antimicrobial agents has risen up the bacteriophage therapy research through out the world. Rather than using antibiotics, it is suggested that the bacterial infection is treated by the administration of bacteriophages. The research was performed in order to characterise the bacteriophages against the pathogenic bacteria *Escherichia coli* (*E. coli*) that have been identified as the causing agent of the colibacillosis in chicken. The host bacteria of the bacteriophages were *E. coli* strains 712X, 141 1E, 141 2E, and 141 3E. All of these *E. coli* strains were serotype O1:K1 which is causative agent for colibacillosis in chicken. There are two bacteriophages that are been characterised which are ØMS1 and ØMS2. All the bacteriophages were characterised using physiological characterisation. Both bacteriophages show their lytic ability toward *E. coli* strains 712X only. It is shown that ØMS1 is better in comparison of ØMS2. Both ØMS1 and ØMS2 demonstrated an optimum multiplicity of infection (MOI) of 0.01 and 0.1 respectively. Based on adsorption rate, ØMS1 reach it maximum value (99.8%) at 8 minutes while ØMS2 reach it maximum value (99.6%) at 10 minutes. In the single step growth curve of the bacteriophages, ØMS1 have longer latent period (25 min) compare to ØMS2 (15 min), with higher burst size of 200 bacteriophage particles per infected cell and 175 bacteriophage particles per infected cell respectively. Both ØMS1 and ØMS2 are stable in temperature range of 37-40 °C with pH range of 6-9.

ABSTRAK

Kemunculan bakteria patogenik yang tahan terhadap kebanyakan ejen antimikrob telah meningkatkan penyelidikan terapi bakteriofaj di seluruh dunia. Daripada menggunakan antibiotik, adalah dicadangkan bahawa jangkitan bakteria dirawat dengan penggunaan bakteriofaj. Kajian ini dilakukan untuk mencirikan bakteriofaj terhadap bakteria patogenik *Escherichia coli* (*E. coli*) yang telah dikenal pasti sebagai agen yang menyebabkan colibacillosis kepada ayam. Organisma ujian untuk bakteriofaj dikenalpasti sebagai bakteria *E. coli* strain 712X, 141 1E, 2E 141, dan 141 3E. Semua strain *E. coli* ini adalah serotype O1: K1 yang merupakan agen penyebab untuk colibacillosis dalam ayam. Terdapat dua bakteriofaj yang telah dicirikan iaitu ØMS1 dan ØMS2. Semua bakteriofaj telah dicirikan menggunakan pencirian fisiologi. Kedua-dua bakteriofaj menunjukkan keupayaan 'lytic' mereka terhadap *E. coli* strain 712X sahaja. MOI optimum untuk kedua-dua ØMS1 dan ØMS2 adalah 0.01 dan 0.1 masing-masing. Daripada kajian, ia menunjukkan bahawa ØMS1 adalah lebih baik berbanding daripada ØMS2. Berdasarkan kadar penjerapan, nilai maksimum (99.8%) ØMS1 pada 8 minit walhal nilai maksimum (99.6%) ØMS2 pada 10 minit. Graf 'single-step growth' menunjukkan bahawa ØMS1 mempunyai tempoh 'latent' yang lebih lama (25 min) berbanding ØMS2 (15 min), dengan saiz 'burst' lebih tinggi 200 zarah bakteriofaj setiap sel yang dijangkiti dan 175 zarah bakteriofaj setiap sel dijangkiti masing-masing. Kedua-dua ØMS1 dan ØMS2 stabil dalam suhu 37-40 °C dengan pH 6-9.

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TABLE OF CONTENTS

PENGESAHAN	i
ABSTRACT	ii
<i>ABSTRAK</i>	iii
ACKNOWLEDGEMENT	iv
LIST OF ABBREVIATION	vii
LIST OF TABLES	viii
LIST OF FIGURES	ix
CHAPTER 1	1
INTRODUCTION	1
CHAPTER 2	3
LITERATURE REVIEW	3
2.1 Escherichia coli (<i>E. coli</i>)	3
2.1.1 Role of <i>E. coli</i> in Poultry Industry	4
2.1.2 APEC in Colibacillosis	5
2.2 The Antibiotics in Livestocks Production	7
2.3 Bacteriophages	9
2.3.1 Introduction	9
2.4 Bacteriophage Therapy	10
CHAPTER 3	14
MATERIALS AND METHODS	14
3.1 Media Preparation	14
3.2 Chemical Preparation	14
3.3 Bacteria Preparation	14
3.3.1 Growth and Maintenance of Bacteria	14
3.3.2 Bacteria Growth Curve	14
3.3.3 Bacteria Concentration	15
3.4 Physiological Characterisation of Bacteriophage	15
3.4.1 Growth and Maintenance of Bacteriophages	15
3.4.2 Determination of Bacteriophage Titre	15
3.4.3 Host Range Test	16
3.4.4 Multiplicity of Infection (MOI)	16
3.4.5 Adsorption rate of Bacteriophage	17
3.4.6 Single-Step Growth Curve	17
3.4.7 Determination of Optimum pH	18
3.4.8 Determination of Optimum Temperature	18
CHAPTER 4	19
RESULTS AND DISCUSSION	19
4.1 Growth Curve of Bacteria	19
4.2 Physiological Characterisation of Bacteriophage	20
4.2.1 Host Range Determination	20
4.2.2 Multiplicity of Infection (MOI)	21
4.2.3 Single-Step Growth Curve	25

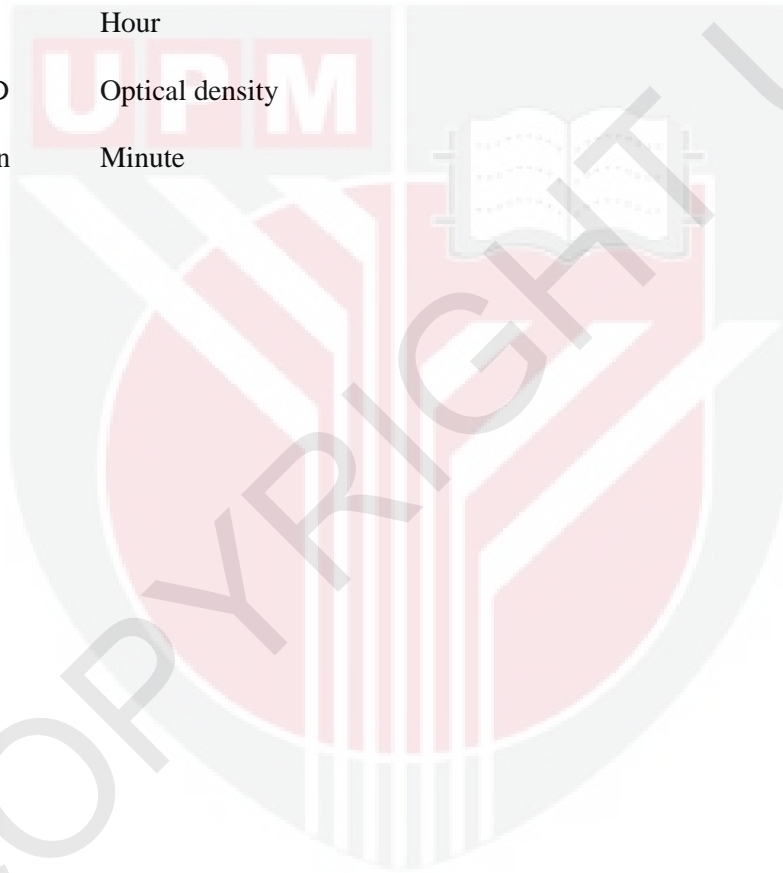
4.2.4	Adsorption Rate of Bacteriophage	27
4.2.5	Determination of Optimum pH	28
4.2.6	Determination of Optimum Temperature	32
CHAPTER 5		36
CONCLUSION AND RECOMMENDATIONS		36
REFERENCES		37
APPENDICES		42



LIST OF ABBREVIATION

Abbreviation

ml	mililiter
µl	microliter
g	Gram
%	Percent
°C	Degree Celsius
h	Hour
OD	Optical density
min	Minute



LIST OF TABLES

Table	Caption	Page
1	Growth curve of <i>E. coli</i> strains 712X over time	29



LIST OF FIGURES

Figures	Caption	Page
1	Chicken with symptoms of Colibacillosis.	5
2	Fibrin deposited in chicken carcass	6
3	Growth curve of <i>E. coli</i> strains 712X over time	20
4	Bacteriophage titre of ØMS1 and bacteria (<i>E. coli</i> strain 712X) concentration over time for different MOIs (0.01, 0.1 and 1)	23
5	Bacteriophage titre of ØMS2 and bacteria (<i>E. coli</i> strain 712X) concentration over time for different MOIs (0.01, 0.1 and 1)	25
6	Single-step growth curve of ØMS1 and ØMS2	27
7	The adsorption rate (%) of ØMS1 and ØMS2 over time.	28
8	The lytic ability of ØMS1 and ØMS2 over time at OD _{600nm}	29
9	Determination of optimum pH for lytic activity of ØMS1	30
10	Determination of optimum pH for lytic activity of ØMS2	32
11	Determination of optimum temperature for lytic activity of ØMS1:	34
12	Determination of optimum temperature for lytic activity of ØMS2:	36

CHAPTER 1

INTRODUCTION

Avian pathogenic *Escherichia coli* (APEC) caused a huge economic damage to the poultry industry due to high mortality, slower growth, a lower feed conversion efficiency and condemnation of carcasses at the abattoir. Inhalation of dust contaminated with high concentrations of *E. coli* can infect the air sacs and lungs, bacteria can enter the bloodstream, causing systemic disease known as colibacillosis.

Colibacillosis is a disease of severe economic significance to all poultry producers worldwide and is characterized by a diverse array of lesions. Recent reports in Western Europe implicate a resurgence of this disease in the poultry industry, particularly in chicken layers (Zanella et al., 2000; Vandekerchove et al., 2004; Jordan et al., 2005). Depending on the virulence status of the strain, host status and presence and type of predisposing factors, the infection manifests as an initial septicaemia that is followed by either sudden death or localized inflammation in multiple organs. The disease is traditionally treated with antibiotics, but due to frequent use of antibiotics many APEC strains have become multidrug resistant.

Normally, disinfectants are used in poultry houses to prevent colibacillosis and other diseases carry-over between sequential flocks, to lower the number of specific pathogens as much as possible. Furthermore, it was determined if these APEC isolates were resistant against the most included active ingredients in routinely used disinfectants in the poultry industry. High resistance percentages were found against antibiotics frequently used in the poultry industry such as ampicillin,

nalidixic acid, sulfonamides and trimethoprim. The use of antibiotics raises a public health concern, due to transfer of resistance genes or resistant bacteria from animals to humans. Therefore the use of bacteriophage therapy was tested as alternative treatment for colibacillosis in chickens.

Bacteriophages are viruses that infect and kill bacteria. Bacteriophages kill bacteria, which provide an opportunity to use bacteriophage as an alternative to antibiotics to prevent and treat bacterial infections and to reduce foodborne pathogens on agricultural products.

The general objective of this study is to characterise bacteriophages for the *E. coli* causing colibacillosis in chicken. The specific objectives are as follows:

1. To study the growth kinetics of the *E. coli* causing colibacillosis in chickens.
2. To characterise the bacteriophage isolated against *E. coli* causing colibacillosis in chickens.

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