



Faculty of Medicine and Health Sciences

**Isolation and Characterisation of Bacteriophages infecting
Klebsiella pneumoniae from Sewage Samples in Sarawak, Malaysia**

Andrea Ken Paran

**Master of Science
2020**

Isolation and Characterisation of Bacteriophages infecting
Klebsiella pneumoniae from Sewage Samples in Sarawak, Malaysia

Andrea Ken Paran

A thesis submitted

In fulfillment of the requirements for the degree of Master of Science

(Medical Microbiology)

Faculty of Medicine and Health Sciences
UNIVERSITI MALAYSIA SARAWAK

2020

DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.



.....
Signature

Name: Andrea Ken Paran

Matric No.: 17020020

Faculty of Medicine and Health Sciences

Universiti Malaysia Sarawak

Date : 20th May 2020

ACKNOWLEDGEMENT

Firstly, I would like expressed my gratitude to my supervisor Dr. Tan Cheng Siang of Faculty of Medicine and Health Sciences, UNIMAS for his continuous guidance, steering me in the right direction throughout my research and writing.

I would also like to acknowledge Graduate School of UNIMAS for awarding me the Zamalah scholarship to support me in my studies of which I am truly grateful for and this research has been funded by F05/SpGS/1564/2017 grant.

Lastly, I would like to express my profound gratitude to my parents and friends for providing me with unfailing support and continuous encouragement throughout my years of study and through the process of researching and writing this thesis. This accomplishment would not have been possible without them.

Thank you.

Jeremiah 29:11

“For I know the plans I have for you,” declares the Lord, “plans to prosper you and not to harm you, plans to give you hope and a future.

ABSTRACT

A group of nosocomial multidrug-resistant pathogens that posed threats to immunocompromised patients have previously been referred to by the acronym ESKAPE. The ESKAPE pathogens which include Gram-negative *Klebsiella pneumoniae* are considered a great threat, due to the emergence of strains that are resistant to all or most available antibiotics. Misuse and extensive consumption of broad-spectrum antibiotics in hospitalised patients have allowed the evolution of drug-resistant bacterial strains by producing defense mechanisms such as extended-spectrum β -lactamases and diverse aminoglycoside-inactivating enzymes. Many of these strains are highly virulent and exhibit a strong tendency to propagate. In this study, five lytic *Klebsiella* bacteriophages namely ϕ KPaV03, ϕ KPaV04, ϕ KPaV08, ϕ KPaV10, and ϕ KPaV12 were isolated from domestic sewage at Universiti Malaysia Sarawak (UNIMAS) and characterised based on their biological properties, including bacteriophage morphology, host range, growth curve, bacteriophage multiplicity of infection (MOI) and structural protein composition. These bacteriophages have large burst size with high titer assay between $10^8 - 10^{12}$ pfu / mL and were predominantly stable under 4 °C. Two among the five bacteriophages were capable of efficiently lysing more than five *Klebsiella pneumoniae* strains out of 18 clinical and community-acquired isolates from Borneo Medical Centre (BMC) and students of UNIMAS, respectively. These bacteriophages exhibit several properties indicative of potential utility in phage cocktails and phage-antibiotic synergy (PAS) approach.

Keywords: *Klebsiella pneumoniae*, multidrug-resistant, sewage samples, bacteriophages, phage approaches

Pengasingan dan Pengenalpastian Bakteriofaj menjangkiti Klebsiella pneumoniae dari Sampel Kumbahan di Sarawak, Malaysia

ABSTRAK

Sekumpulan patogen nosokomial yang resisten terhadap pelbagai jenis antibiotik telah menimbulkan ancaman kepada pesakit yang lemah daya tahan. Patogen nosokomial ini telah dirujuk sebagai akronim ESKAPE. Patogen ESKAPE, termasuklah bakteria Gram-negatif Klebsiella pneumoniae yang dianggap sebagai ancaman besar, kerana resisten dengan kebanyakan antibiotik yang sedia ada. Penyalahgunaan antibiotik spektrum luas oleh pesakit-pesakit telah menyebabkan evolusi bakteria yang resisten dengan pelbagai jenis antibiotik melalui mekanisme pertahanan seperti spektrum lanjutan β -laktamase dan enzim yang menyah-aktif aminoglikosida. Kebanyakan strain bakteria Klebsiella pneumoniae menunjukkan daya virulen yang tinggi. Kajian ini telah berjaya memencilkan lima bakteriofaj Klebsiella iaitu ϕ KPaV03, ϕ KPaV04, ϕ KPaV08, ϕ KPaV10, dan ϕ KPaV12 dari sampel kumbahan domestik Universiti Malaysia Sarawak (UNIMAS) dan dikategorikan berdasarkan sifat biologi, termasuk morfologi bakteriofaj, kesan jangkitan bakteriofaj terhadap pelbagai jenis Klebsiella pneumoniae, lengkung pertumbuhan bakteriofaj, pergandaan jangkitan bakteriofaj (MOI) dan komposisi struktur protein. Kesemua bakteriofaj yang terpilih mempunyai saiz letusan besar dengan kadar titer yang tinggi antara 10^8 - 10^{12} pfu / mL dan kebanyakannya stabil di bawah suhu 4 °C. Dua daripada lima bakteriofaj mampu membasmi lebih daripada lima strain Klebsiella pneumoniae daripada 18 isolat klinikal dan komuniti dari Pusat Perubatan Borneo (BMC) dan pelajar-pelajar

UNIMAS. Disamping itu, bakteriofaj ini juga telah mempamerkan beberapa sifat yang menunjukkan potensi kegunaan dalam koktel bakteriofaj dan juga secara pendekatan sinergi bakteriofaj-antibiotik (PAS).

Kata kunci: *Klebsiella pneumoniae, resisten pelbagai antibiotik, sampel kumbahan, bakteriofaj, pendekatan bakteriofaj*

TABLE OF CONTENTS

	Page
DECLARATION	i
ACKNOWLEDGEMENT	ii
ABSTRACT	iii
<i>ABSTRAK</i>	iv
TABLE OF CONTENTS	vi
LIST OF TABLES	xii
LIST OF FIGURES	xiv
LIST OF ABBREVIATIONS	xvii
CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW	1
1.1 General Introduction	1
1.2 The History of Therapeutic Bacteriophages	3
1.3 Emergence of Antibiotic-Resistant Bacteria	4
1.4 <i>Klebsiella pneumoniae</i> in a Healthcare Setting	5
1.5 Antibiotic Resistance Mechanism of <i>Klebsiella pneumoniae</i>	7
1.5.1 Prevalence of Antibiotic-Resistant <i>Klebsiella pneumoniae</i> in Malaysia	9
1.6 Bacteriophage Therapy	10
1.6.1 Type of Bacteriophages	10
1.6.2 Bacteriophages Life Cycle	12

1.6.3	Pros and Cons of Phages as Antimicrobials	14
1.6.4	Potentials, Safety and Current Clinical Trials of Therapeutic Bacteriophages	16
1.6.5	Isolated Bacteriophages Infecting <i>Klebsiella pneumoniae</i>	18
1.7	Study Objectives	20
CHAPTER 2: MATERIALS AND METHODS		21
2.1	Culture Medium Sterilisation	21
2.1.1	Filter Sterilisation	21
2.1.2	Heat Sterilisation	21
2.2	Culture Media and Reagents Preparation	21
2.2.1	Preparation of Media Broth – Luria-Bertani (LB) Broth	21
2.2.2	Preparation of Media Agar – Luria-Bertani (LB) Agar	21
2.2.3	Preparation of Media Agar – Mueller-Hinton (MH) Agar	22
2.2.4	Preparation of Glycerol Solution (50 % v/v)	22
2.2.5	Preparation of SM Buffer	22
2.2.6	Preparation of Phosphate Buffered Saline (PBS) Buffer	22
2.2.7	Preparation of 1X Tris-Acetate EDTA (TAE) Buffer	23
2.2.8	Preparation of 0.1M Calcium Chloride (CaCl ₂) Solution	23
2.3	Antibiotic used for Antibiotic Sensitivity Testing (AST)	23
2.4	Bacterial Strains	24
2.5	Preparation of <i>Klebsiella</i> spp. Log-phase Culture Broth	25

2.6	Verification of <i>Klebsiella pneumoniae</i> Genomic DNA	26
2.6.1	Wizard® Genomic DNA Purification Kit by Promega	26
2.6.2	Bacterial 16S rRNA PCR primers	26
2.6.3	Bacterial 16S rRNA PCR Condition	27
2.6.4	Agarose Gel Electrophoresis	27
2.6.5	Bacterial 16S rRNA Sequencing	28
2.7	Antibiotic Sensitivity Testing on <i>Klebsiella pneumoniae</i> Community-acquired Isolates	28
2.8	Housekeeping Genes Sequencing of <i>Klebsiella pneumoniae</i>	28
2.8.1	Housekeeping PCR primers	29
2.9	Bacteriophages Isolation, Purification, and Titration	30
2.9.1	Bacteriophages Sample Collection	30
2.9.2	Bacteriophages Enrichment	31
2.9.3	Bacteriophages Serial Dilution	32
2.9.4	Plaque Assay – Double-layer Agar Overlay Technique	32
2.9.5	Identifying and Verifying Putative Plaques (Spot Assay)	32
2.9.6	Bacteriophage Titration	33
2.10	Bacteriophages Characterisation	33
2.10.1	Phage Host Range Analysis	33
2.10.2	Step-by-Step (SBS) Phage Isolation Method	34

2.10.3	Single-step Growth Curve	34
2.10.4	Multiplicity of Infection (MOI)	35
2.10.5	High Titer Stock of Purified Bacteriophages	35
2.10.6	Bacteriophage DNA Extraction	35
2.10.7	Characterisation of Genes Encoding Bacteriophage Proteins	36
2.11	Phage Cocktail and Phage Antibiotic Synergistic against Antibiotic-Resistant <i>Klebsiella pneumoniae</i>	39
2.11.1	Phage Cocktail	39
2.11.2	Minimum Inhibitory Concentration (MIC) Selection	40
2.11.3	Phage-Antibiotic Synergy (PAS)	41
CHAPTER 3:	RESULTS	43
3.1	Verification of <i>Klebsiella pneumoniae</i> Genomic DNA	43
3.1.1	DNA Fragments Visualisation by Agarose Gel Electrophoresis	43
3.1.2	NCBI BLAST® Analysis	44
3.2	Determination on <i>Klebsiella</i> spp. Log-phase	45
3.3	Antibiotic Sensitivity Testing (AST) on <i>Klebsiella pneumoniae</i> Clinical Isolates	46
3.4	Housekeeping Genes Sequencing Analysis of <i>Klebsiella pneumoniae</i>	49
3.5	Bacteriophages Isolation, Purification and Titration	54
3.5.1	Bacteriophages Serial Dilution	54
3.5.2	Step-by-Step (SBS) Method	55

3.5.3	Bacteriophage Host Range Analysis	56
3.5.4	Bacteriophage Titer	59
3.6	Bacteriophage Characterisation	59
3.6.1	Plaque Morphology	59
3.6.2	Multiplicity of Infection (MOI)	61
3.6.3	Single-Step Growth Curve	61
3.6.4	Bacteriophages Structural Protein Extraction	64
3.6.5	Bacteriophage Structural Protein Sequencing Analysis by UniProt Blast	68
3.7	Phage Cocktail and Phage-Antibiotic Synergy Approaches against Antibiotic Resistant <i>Klebsiella pneumoniae</i>	69
3.7.1	Phage Cocktail	69
3.7.2	Minimum Inhibitory Concentration (MIC) Selection	70
3.7.3	Comparison of Phage Cocktail and Phage-Antibiotic Synergy Approaches	71
CHAPTER 4: DISCUSSION		75
4.1	Verification of <i>Klebsiella pneumoniae</i>	75
4.2	Housekeeping Genes Sequencing Analysis	76
4.3	Antibiotic Sensitivity Test	78
4.4	Bacteriophage Isolation	79
4.5	Bacteriophages Plaque Morphologies	80
4.6	Host Range	80

4.7	Single-step Phage Growth Curve	81
4.8	Bacteriophage Structural Proteins Analysis	82
4.9	Minimum Inhibitory Concentration (MIC)	83
4.10	Phage Cocktail	84
4.11	Phage-Antibiotic Synergy (PAS) Approaches	85
CHAPTER 5: CONCLUSION AND FUTURE STUDIES		87
REFERENCES		89
APPENDICES		105

LIST OF TABLES

	Page
Table 2.1 List of antimicrobial agents used against <i>Klebsiella pneumoniae</i>	23
Table 2.2 <i>Klebsiella</i> spp. isolates used throughout the experiment	24
Table 2.3 Components of <i>Klebsiella pneumoniae</i> 16S rRNA PCR mixture in 50 μ L reaction	27
Table 2.4 Universal sequencing primers for <i>Klebsiella pneumoniae</i> housekeeping genes	29
Table 2.5 Components of <i>Klebsiella pneumoniae</i> housekeeping genes PCR mixture in 25 μ L reaction	30
Table 2.6 PCR cycle condition for 25 μ L of <i>Klebsiella pneumoniae</i> housekeeping genes	30
Table 2.7 Specific primers used for Phage Structural Protein analysis	37
Table 2.8 Components of <i>Klebsiella</i> phage structural proteins PCR mixture in 30 μ L reaction	37
Table 2.9 PCR condition for Lysin gene	38
Table 2.10 PCR condition for Viral Capsid Assembly protein gene (g20)	38
Table 2.11 PCR condition for Major Capsid protein gene (g23)	38
Table 2.12 PCR condition for Tail Fiber protein gene (T1)	39

Table 3.1	The percentage of BLAST similarity of <i>Klebsiella</i> spp. 16S rRNA PCR sequences	45
Table 3.2	Antibiotics sensitivity testing of <i>Klebsiella pneumoniae</i> clinical strains (CRKP1 to KP9)	48
Table 3.3	Antibiotics sensitivity testing of <i>Klebsiella pneumoniae</i> clinical and non-clinical strains (KP10 to ATCC KP)	49
Table 3.4	Sequence query of <i>Klebsiella</i> locus under MLST scheme	50
Table 3.5	Bacteriophages isolated using different host strains respectively by SBS method	56
Table 3.6	Assessment of phages host range by spot assay	58
Table 3.7	Average titer (pfu / mL) of phage isolates	59
Table 3.8	Plaque assay analysis of <i>Klebsiella pneumoniae</i> clinical strains infected with increasing doses of isolated <i>Klebsiella</i> phages dilution (MOI)	61
Table 3.9	Summary of amplification of each isolated phage structural protein genes	64
Table 3.10	Characteristics of sequenced <i>Klebsiella</i> phages	68
Table 3.11	Minimum inhibitory concentration (MIC) of Gentamicin-resistant <i>Klebsiella pneumoniae</i> strains	71

LIST OF FIGURES

	Page
Figure 1.1	Electron microscope images of three families of tailed phages 11
Figure 1.2	The actions of lytic phages as bacterial predators achieve several steps to attain host destruction 13
Figure 2.1	Location of sampling site in Sarawak with GPS coordinates 31
Figure 3.1	Agarose gel electrophoresis of PCR product for universal primers 27F and 152R 43
Figure 3.2	Agarose gel electrophoresis of PCR product for universal primers 27F and 152R 44
Figure 3.3	Growth curve of ATCC KP within 24 h using OD600 measurements. Each point corresponds to the average of three determinations $\pm$ standard deviation 46
Figure 3.4	Antibiotic sensitivity test - Different antibiotics tested on KP15 47
Figure 3.5	Phylogenetic tree resulting from Maximum Likelihood Method for <i>Klebsiella pneumoniae</i> rpoB gene using Mega 6 software 51
Figure 3.6	Phylogenetic tree resulting from Maximum Likelihood Method for <i>Klebsiella pneumoniae</i> gapA gene using Mega 6 software 52
Figure 3.7	Phylogenetic tree resulting from Maximum Likelihood Method for <i>Klebsiella pneumoniae</i> tonB gene using Mega 6 software 53

Figure 3.8	Three different phage plaque morphologies (A, B and C) infecting host KP17	55
Figure 3.9	Spot assay of different phages spotted on bacterial host KP10	57
Figure 3.10	Different plaque morphologies formed by isolated phages infecting <i>Klebsiella pneumoniae</i> strains from sewage influent	60
Figure 3.11	The single-step growth curve assay for phage ϕ KPaV03 infecting ATCC KP at MOI 1. The latent period takes less than 20 mins.	62
Figure 3.12	The single-step growth curve assay for phage ϕ KPaV04 infecting CRKP1 at MOI 10. The latent period takes approximately 60 mins.	62
Figure 3.13	The one-step growth curve assay for phage ϕ KPaV08 infecting KP17 at MOI 0.01. The latent period takes less than 5 mins.	63
Figure 3.14	The one-step growth curve assay for phage ϕ KPaV10 infecting ATCC KP at MOI 0.1. The latent period takes approximately 20 mins.	63
Figure 3.15	The one-step growth curve assay for phage ϕ KPaV12 infecting KP15 at MOI 0.01. The latent period takes less than 10 mins.	64
Figure 3.16	PCR products of Lysin gene (Lys)	65
Figure 3.17	PCR products of Major Capsid Protein gene (g23)	66
Figure 3.18	PCR products of Tail Fiber gene (T1)	67
Figure 3.19	Phage cocktail approach against single <i>Klebsiella</i> spp. strains	69

Figure 3.20	Time-kill curves of phage cocktail (PC), phage-antibiotic synergy when treated with sub-inhibitory concentration of Gentamicin (PA) and growth control curve (GC) versus ATCC KP	72
Figure 3.21	Time-kill curves of phage cocktail (PC), phage-antibiotic synergy when treated with sub-inhibitory concentration of Gentamicin (PA) and growth control curve (GC) versus CRKP1	72
Figure 3.22	Time-kill curves of phage cocktail (PC), phage-antibiotic synergy when treated with sub-inhibitory concentration of Gentamicin (PA) and growth control curve (GC) versus ESKP2	73
Figure 3.23	Time-kill curves of phage cocktail (PC), phage-antibiotic synergy when treated with sub-inhibitory concentration of Gentamicin (PA) and growth control curve (GC) versus ESKP3	73
Figure 3.24	Time-kill curves of phage cocktail (PC), phage-antibiotic synergy when treated with sub-inhibitory concentration of Gentamicin (PA) and growth control curve (GC) versus KP5	74
Figure 3.25	Time-kill curves of phage cocktail (PC), phage-antibiotic synergy when treated with sub-inhibitory concentration of Gentamicin (PA) and growth control curve (GC) versus KP9	74

LIST OF ABBREVIATIONS

BLAST	Basic Local Alignment Search Tool
blaKPC	<i>Klebsiella pneumoniae</i> Carbapenemase
CAP	Community-acquired Pathogens
CO ₂	Carbon Dioxide
CFU	Colony Forming Units
DNA	Deoxyribonucleic Acid
dsDNA	Double-stranded Deoxyribonucleic Acid
g	Gram
ICTV	International Committee on Taxonomy of Viruses
kb	Kilobase
LB	Luria-Bertani
MDR	Multi-drug Resistant
mL	Milliliter
mm	Millimeter
mg	Milligram
MOI	Multiplicity of Infection
NDM	New Delhi Metallo- β -Lactamase
nm	Nanometer
OD	Optical Density
RNA	Ribonucleic Acid
rpm	Rotation per Minute
pmol	Picomole
PCR	Polymerase Chain Reaction

PFU	Plaques per Unit
ssDNA	Single-stranded Deoxyribonucleic Acid
ssRNA	Single-stranded Ribonucleic Acid
TAE	Tris-Acetate-EDTA
TEM	Transmission Electron Microscope
V	Voltage
v/v	Volume per Volume
μL	Microlitre

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 General Introduction

The bacteriophages were independently discovered by British microbiologist Frederick Twort in 1915 and French-Canadian microbiologist Felix d'Hérelle in 1917 (Carlton, 1999). A discovery which occurred about 20 years before the practical application of penicillin, the first antibiotic (Matsuzaki et al., 2005). At the time of discovery, bacteriophages were regarded as a potential treatment for bacterial infections and have been developed to control bacterial diseases such as dysentery, cholera, and gangrene (Dublanchet & Bourne, 2007).

Regardless of the attributes and unique properties of bacteriophages being able to fight bacterial infections, it was then soon abandoned in the 1940s by the West with the arrival of the antibiotic era (Matsuzaki et al., 2005). With the rise of pharmaceutical antibiotics in the mid-20th century, along with a better understanding of diseases and sanitation, both quality of life and life expectancy in the industrialized world drastically improved (Lin et al., 2017). Antibiotics rapidly became an indispensable medical tool with millions of kilograms used globally each year in the prophylaxis and treatment of people, animal, and agriculture (Levy & Bonnie, 2004).

However, the excessive usage and prescription of antibiotics leads to the emergence of pathogenic bacteria resistant to multiple antimicrobial agents such as the ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.) (Weber-

Dabrowska et al., 2016). Antibiotic-resistant genes encoding for resistance to common antibiotics, including β -lactams, aminoglycosides, and tetracycline, are posing a major threat to current medical treatment of common diseases, and these genes now appear to be abundant in the environment (Lin et al., 2017).

The growing of multidrug-resistant pathogens threatens to make the revolutionary achievements of modern medicine obsolete, posing a concern of re-entering the “pre-antibiotics” era (Jeney, 2012). For example, Lin et al., (2017) mentioned the lack of treatment options for hospital-acquired carbapenem-resistant *K. pneumoniae* infections caused a 40 % - 50 % mortality rate in the United States beginning of 2000. Carbapenem resistance in *K. pneumoniae*, mainly attributed to the production of *K. pneumoniae* carbapenemase (KPC) enzyme which is able to destroy carbapenems and causes resistance against a wide spectrum of antibiotics.

Rekindling phage therapy can be a good option for solving the problem of multidrug-resistant pathogens such as *K. pneumoniae*. Major advantages of phage therapy compared to conventional antibiotic treatment are such that phages are highly specific and easy to obtain. Thus, it can be used in cases of sudden bacterial disease outbreaks. Moreover, they do not have chemical side effects like antibiotics (Baharuddin et al., 2017).

Therefore, the objectives of this study were to isolate and characterise lytic bacteriophages with therapeutic potential against clinical- and community-acquired *K. pneumoniae* strains. It is hoped that this study will be able to contribute to future phage therapy studies and applications.

1.2 The History of Therapeutic Bacteriophages

Bacteriophage, or simply called as phages, are viruses that specifically infect and lyse bacteria. The term bacteriophage was coined by Felix d'Herelle in 1916, who derived it from the word 'bacteria' and a Greek word 'phagein' which means 'to devour'. Phages, like all other viruses, are absolute parasites as they have no machinery to generate energy and ribosomes for making proteins even though they carry all the information to direct their own reproduction in an appropriate host. (Kutter & Sulakvelidze, 2005).

In 1910, d'Herelle first observed the bacteriophage phenomenon while studying microbiologic means of controlling an epizootic locusts in Mexico which he then applied his knowledge on phage to treat the dysentery outbreak among several French soldiers (Sulakvelidze, Alavidze, & Morris Jr., 2001). Following the success, the usage of bacteriophage as a therapeutic agent to treat bacterial infections has been widely applied circa the 1930s and 1940s in Eastern Europe and the former Soviet Union. However, it was abandoned by Western Medicine in the 1940s after the discovery of penicillin which soon became widely available (Matsuzaki et al., 2005).

The reason for abandoning the therapeutic usage of phages in the West was due to the lack of appropriate controls and inconsistent results (Wittebole et al., 2014). Moreover, at that time, the biological viral nature and mechanism of phage has yet to be determined and thoroughly studied until they were visualised in the 1940s after the invention of electron microscopy (Cisek et al., 2017).

Merril et. al., (2003) stated that the applications of phage as practiced in the Soviet Union including Poland have been extensively evaluated in which according to one of the review papers from 1998, only 27 papers dealing with bacteriophage therapy were published

between 1966 and 1996. Nineteen of these were from laboratories in Poland and Russia, where research on bacteriophage therapy had never dimmed, and where patients infected with *Staphylococcus*, *Streptococcus*, *Klebsiella*, *Escherichia*, *Proteus*, *Pseudomonas*, *Shigella*, and *Salmonella* were reportedly treated with 80 to 85 percent success (Ho, 2001). Likewise, Bull et al., (2002) has reviewed on the earlier experimental work on phage therapy and prophylaxis and mentioned several impressive studies using phage to treat and prevent bacterial infections in animals including work done by Williams S. H. and team in 1987.

However, details such as the primary qualitative data such as phage dosages and clinical criteria were not properly reported and documented. Hence, most of the studies from Eastern Europe are unable to meet the present standards for pharmaceutical approval in countries that require certification based on the results of efficacy and pharmacokinetic studies in animals and humans.

1.3 Emergence of Antibiotic-Resistant Bacteria

Since the discovery of penicillin by Alexander Fleming in 1928, antibiotics were successfully used to treat bacterial infections in humans and animals, as well as in agriculture. However, the effectiveness of antibiotics is challenged by the increasing number of antibiotic-resistant bacteria. Most of the available antibiotics, including β -lactams, are becoming less effective and in some cases, resistance rates exceed 98 % (Akinkunmi et al., 2014). For instance, El-Shibiny & El-Sahhar (2017) have reported on few multidrug-resistant strains such as *Escherichia coli* O104:H4 and some *Salmonella* isolates from poultry which have been found to be resistant to at least 14 different antibiotics, and about 90 % of the *Salmonella* isolates were found to be resistant to at least one or more antibiotics tested.

A group of hospital-acquired multidrug-resistant nosocomial pathogens that posed threat to immunocompromised patients have previously been referred to by the acronym ESKAPE, which includes Gram-positive *Enterococcus faecium* and *Staphylococcus aureus*, as well as Gram-negative *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* species (Navidinia, 2008; Viertel et al., 2014; Santajit & Indrawattana, 2016). Methicillin-resistant *Staphylococcus aureus* (MRSA) was found to be resistant to many classes of antibiotics, including the 4th-generation fluoroquinolones, and can cause systemic infections with a mortality rate of 50 % (Chang et al., 2016).

Several literatures have reported on the increase of resistance among many medically important bacteria over time due to the misuse of antibiotics and will ultimately increase the cost of treatment, prolong the illness, and increase the rate of mortality (El-Shibiny & El-Sahhar, 2017). As it is, more than half a million people worldwide die each year from antibiotic-resistant bacterial infections. Therefore, the interest in phage therapy has been revived in Western countries now that the number of antibiotic-resistant bacteria is rapidly growing, especially after the US National Institute of Allergy and Infectious Diseases listed phage therapy as one of 7 strategies to fight antibiotic resistance.

1.4 *Klebsiella pneumoniae* in a Healthcare Setting

The Gram-negative ESKAPE pathogens which include Gram-negative *Klebsiella pneumoniae* are considered the greatest threat, due to the emergence of strains that are resistant to all or most available antibiotics (Wyres & Holt, 2018). *K. pneumoniae* belonged to the family of *Enterobacteriaceae* and are frequently considered opportunistic pathogens causing hospital-acquired infections such as pneumonia, urinary tract infections (UTI) and pyogenic liver abscess (Chung, 2016; Clegg & Murphy, 2016; Holt et al., 2015).

Kidd et al., (2017) stated that *K. pneumoniae* being one of the multidrug-resistant (MDR) organisms was discerned as an emergency threat to the human health by the World Health Organisation, the US Centers for Disease Control and Prevention and the UK Department of Health.

The most prevalent type of nosocomial infection caused by *K. pneumoniae* is UTI, with 97 % associated with the presence of indwelling urinary catheters and about 80 % of the nosocomial infections caused by *K. pneumoniae* are due to these multidrug-resistant strains (Wang et al., 2015). The Chinese Ministry of Health National Antimicrobial Resistance Investigation Net annual reports estimated that the isolation rate of *K. pneumoniae*, which has become one of the most common pathogenic bacteria in bloodstream infections (BSIs), was 7.7 % and 8.3 % in the year 2010 and 2011, respectively (Zhao et al., 2012).

Cao et al., (2015) mentioned on pneumonia infection caused by *K. pneumoniae* is usually associated with a high mortality rate. In fact, despite the development of broad-spectrum antibiotics, mortality rates greater than 50 % have been reported in those infected with *K. pneumoniae*.

The extensive spread of multidrug-resistant strains of *Klebsiella* spp., including those coding for extended-spectrum β -lactamases (ESBLs) carbapenems and diverse aminoglycoside-inactivating enzymes, has renewed global interest in these pathogens (Karumidze et al., 2012). For example, Bush (2010) reported on surveillance studies conducted in Brooklyn, New York hospitals in 2004 found that 35–40 % of patients in the intensive care unit (ICU) carried carbapenem-resistant isolates of *K. pneumoniae*.

1.5 Antibiotic Resistance Mechanism of *Klebsiella pneumoniae*

To survive within the human host, bacteria have developed several lines of counterattack and evasion strategies. Like many other opportunistic pathogens, *K. pneumoniae* has various virulence factors that help the bacterium survive within the host (Podschun & Ullmann, 1998).

Antibiotic inactivation in *Enterobacteriaceae* is frequently associated with plasmid resistance determinants that are easily transferred among species by bacterial chromosome, plasmid, or transposons (Santajit & Indrawattana, 2016). High-level antibiotic resistance is frequently linked to the transfer of large plasmids that can acquire numerous resistance genes, including genes for multiple β -lactamases from different functional classes (Bush, 2010).

In Gram-negative organisms such as *K. pneumoniae*, the most common β -lactam resistance mechanism involves hydrolysis of β -lactam ring often found in penicillin, cephalosporins, and carbapenems by β -lactamases resulting in subsequent inactivation of the antibiotics (Santajit & Indrawattana, 2016). The increasing prevalence of carbapenem-resistant *K. pneumoniae* (CRKP), with resistance encoded by *bla*KPC, presents a significant challenge for physicians (Bush et al., 1995). In addition, the emergence of the *K. pneumoniae* super enzyme, known as NDM-1 and encoded by *bla*_{NDM-1}, has increased the proportion of carbapenem-resistant *K. pneumoniae* isolates (Yong et al., 2009).

Natural transformation is a predominantly conserved mechanism of horizontal gene transfer in bacterial species that allows the process of evolution and promote the spread of antibiotic resistance determinants, encourage antigenic variation and creates a tendency to the acquisition of novel virulence factors (Ellison et al., 2018). Proven by the recent studies

done by Ellison and colleagues (2018) which showed that the competence type IV pili of *Vibrio cholerae* directly bind to DNA via their tip and mediate DNA internalisation through retraction during the conserved mechanism of horizontal gene transfer. Similarly, fimbriae assembled by the chaperone-usher (CU) assembly pathway produced by *K. pneumoniae* play a role in colonisation and pathogenesis of the urinary tract (Clegg & Murphy, 2016). Chaperone-usher (CU) assembly pathway is often utilised by a broad range of *Enterobacteriaceae* to assemble adhesive surface structures called pili or fimbriae that responsible for bacteria-host cell interactions (Khater et al., 2015).

Another major reasons *K. pneumoniae* possess low antibiotic susceptibility is due to their thick biofilms to reduced cell permeability and are a resistance mechanism against antibiotics (Li et al., 2014). Biofilm formation plays a key role in many bacterial infections and contributes to the ability of bacteria to overcome host-defense mechanisms.

Biofilms produced a matrix of extracellular polymeric substances which allow complex microbial communities to live as a thin layer on biotic or abiotic surfaces. This enables microorganisms within the biofilm to interact with one another, as well as with the environment. The major components of the matrix mainly consist of polysaccharides, proteins, lipids, and extracellular DNA from the microbes. Besides that, the matrix of biofilms also provides a mechanical and biochemical shield that support the conditions needed to incapacitate the activity of the drugs such as low O₂, low pH, high CO₂, and low water availability. Therefore, these conditions created difficulty in eliminating bacteria using conventional antibiotics. Moreover, when the bacteria experience nutrient scarcity, they could become tolerant to antibiotics. This may explain the apparent greater antibiotic resistance of cells in the deep layers of a biofilm (Santajit & Indrawattana, 2016).

1.5.1 Prevalence of Antibiotic-Resistant *Klebsiella pneumoniae* in Malaysia

Based on the study conducted by Loh et al., (2007), *Klebsiella pneumoniae* ranks high as a cause of community-acquired pneumonia (CAP) in hospitalised patients in Malaysia compared to other Southeast Asian countries.

Lim and colleagues (2009) performed molecular characterisation on 51 strains of *K. pneumoniae* isolated from various public hospitals in Malaysia. Majority of the strains with 98 % were susceptible to imipenem whereas 52.9 % were multidrug-resistant (MDR) *K. pneumoniae*. The first report of carbapenem-resistant *K. pneumoniae* in Malaysia was an imipenem resistant strain isolated from the blood culture of a 42-year old woman in 2004 (Low et al., 2017). Subramaniam & Muniandy (2005) have reported on an association of ESBL SHV-5 *K. pneumoniae* with a nosocomial outbreak in a pediatric oncology unit in a Malaysian public hospital.

Al-Marzooq et al., (2015) has reported on a total of 93 Malaysian multi-drug resistant *K. pneumoniae* isolated from patients admitted to University of Malaya Medical Center, Kuala Lumpur, Malaysia between the year 2010-2012 which were examined for antibiotic resistance determinants including extended-spectrum beta-lactamases (ESBLs), aminoglycoside and trimethoprim/sulfamethoxazole resistance genes and plasmid replicons. Their study indicated on the diffusion of highly diverse plasmids with multiple antibiotic resistance determinants among the Malaysian isolates.

For Carbapenem-resistant *Enterobacteriaceae* (CRE) prevalence in Malaysia, a total of 321 *K. pneumoniae* isolates collected during April 2010 - June 2012 from academic hospitals were characterized. Thirteen isolates (4.0 %) were carbapenem-resistant *K.*

pneumoniae (CRKP) and most of them were resistant to all tested antibiotics except colistin and tigecycline (Suwantararat & Carroll, 2016).

1.6 Bacteriophage Therapy

Significant increase in drug-resistant bacteria is generally cited as the principal motivation for rekindled scientific and public interest in the early experiments of the pre-antibiotic era using bacteriophage therapy for bacterial infections, as mentioned previously in Section 1.2. More specifically, the long history of research in bacteriophage therapy makes it a natural alternative to the current and feverish search for new antimicrobials. Furthermore, considering recent arguments that modern genome-based technologies promise to drastically facilitate the development of highly specific bacteriophage, the natural predator of bacteria, seems to be the ultimate target-based antimicrobial agent (Rosamond & Allsop, 2000). A re-visitation of bacteriophage therapy would inadvertently contribute to the knowledge on the nature of the course of bacterial infection and disease.

1.6.1 Type of Bacteriophages

Phages have morphologically evolved into different array of shapes, sizes, capsid symmetries, and structures. Most phages range in size from 24-200 nm in length with T4 being among the largest phages; approximately 200 nm long and 80-100 nm wide. All bacteriophages contain a head structure, which can vary in size and shape. The head encloses nucleic acid and acts as the protective covering. Some phages have tails attached to the phage head. The tail is a hollow tube through which the nucleic acid passes during infection. The base plate and tail fibers are responsible in aiding phage to cohere to the bacterial cell. However, not all phages have base plates and tail fibers (Sridhar, 2006).

Since the development of electron microscope, which led to the recognition of different phage morphologies and aided by genome composition data led to the basis for the phage classification scheme. Bradley (1967) started to classify phage earnestly in his seminal paper that proposed six basic morphological types, corresponding respectively to tailed phages [with contractile tails (1), long and noncontractile tails (2), and short tails (3)], small isometric ssDNA viruses (4), filamentous phages (5), and small ssRNA phages (6). This scheme was adopted by the International Committee on Taxonomy of Viruses (ICTV).

Phages are presently classified in a hierarchical and holistic system with one order and 10 families. Phage particles can be tailed or polyhedral, filamentous or pleomorphic. Over 96 % of the known phages are tailed and contain dsDNA. Tailed phages constitute the order Caudovirales with three families, characterised by contractile, long and noncontractile, or short tails and respectively named as *Myoviridae*, *Siphoviridae*, and *Podoviridae* as shown in Figure 1.1. Their heads are icosahedra or closely related bodies (Ackermann, 2011). Lytic phages of the three families of Caudavirales are the primary focus in phage therapeutic application (Abubakar et al., 2016). The other seven families of cubic, filamentous and pleomorphic phages are small and well defined. They contain double-stranded or single-stranded DNA or RNA.

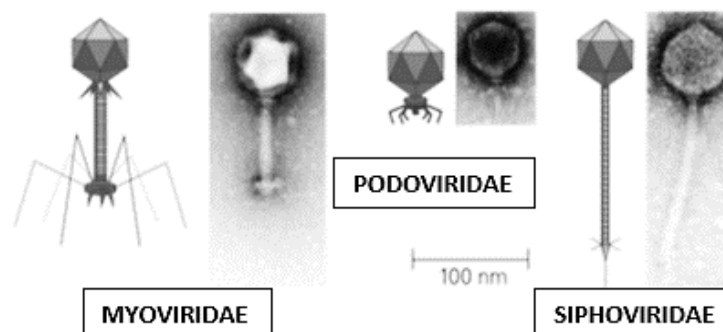


Figure 1.1: Electron microscope images of three families of tailed phages (Harper & Enright, 2011)

1.6.2 Bacteriophages Life Cycle

A common characteristic of phages is that, although their genome carries the information required to drive their own multiplication, they completely rely on the energy and protein biosynthetic machinery of their bacterial hosts to complete their lytic cycle (Demeke et al., 2015). The first contact between phage and its host occurred by random collision, provided that the cell carries specific receptors on its surface. The contact is usually made between the receptor molecules of the host, for example, teichoic acid in Gram-positives or lipopolysaccharide in Gram-negatives and specific phage proteins located at the tip of the tail fiber, or at one end of a filamentous phage. Injection of DNA follows immediately after a phage has stably and irreversibly adsorbed to the cell surface. Based on their subsequent propagation cycle, most phages can be broadly divided into two major groups: virulent and temperate.

Virulent phages immediately redirect the host metabolism toward the production of new phage virions, which are released upon cell death within several minutes to hours after the initial phage attachment event which is known as the lytic cycle. Often in the lytic cycle, virulent phage infection results in clear plaques on their respective host bacterial lawns. Temperate phages can replicate either by the lytic cycle as described above or by establishing a stable long-term viable relationship with their host bacteria. In this state, the phage DNA is replicated together with the host's chromosome. This is termed the lysogenic cycle, during which viral genes that are detrimental to the host are not expressed (Elbreki et al., 2014).

The lytic life cycle occurs when phages kill their hosts by replicating separately from the host's genetic information. During lytic infection, virulent phages inject their nucleic acid into the host cell following attachment as shown in Figure 1.2. Expression of the phage

genome directs the cellular machinery of the host to synthesise new phage macromolecules. The resulting phage progeny are released by fatal cell lysis enabling the lytic cycle to continue as new cells are infected. Stress conditions such as ultraviolet light or chemical mutagens e.g. Mitomycin C can induce a switch to the lytic cycle (Demeke et al., 2015).

Lysogeny or the lysogenic life cycle, on the other hand, is generally associated with temperate phages that can grow either vegetatively or can integrate their genetic material into host chromosomes. In the lysogenic phages, the bacteriophage nucleic acid is integrated into the host bacterium's chromosome. In this condition, the bacterium continues to live and reproduce normally. The genetic material of the bacteriophage, called a prophage, can be transmitted to daughter cells at each subsequent cell division, and later be released either by UV radiation or the presence of certain chemicals, causing proliferation of new phages via the lytic cycle (Demeke et al., 2015).

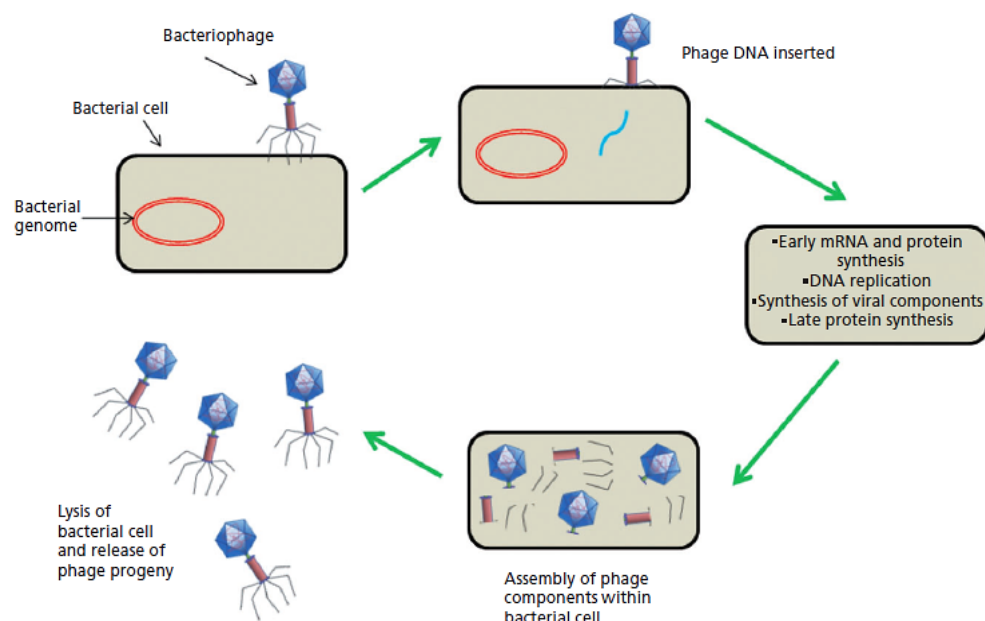


Figure 1.2: The actions of lytic phages as bacterial predators achieve several steps to attain host destruction (Abubakar et al., 2016)

1.6.3 Pros and Cons of Phages as Antimicrobials

When considering phage therapy as a mean to eradicate pathogenic bacteria, there are a few issues that should be considered. Firstly, phage specificity as phages recognise and bind to their hosts by taking advantage of the bacterial cell's surface receptors; therefore, the interactions between phage and bacteria tend to be very specific. Some are so specific, in fact, that they may target only a subgroup of a species, rather than the whole species of bacteria itself (Koskella & Meaden, 2013).

The benefits of specific phage-bacteria interactions unlike broad-spectrum antibiotics minimised the disturbance of the beneficial bacterial microbiota of the body (Loc-Carrillo & Abedon, 2011; Wu et al., 2017). This is important as a healthy and unaltered human microbiome imparts a robust immunity to the host, which may reduce the incidence of preventable diseases associated with a disrupted microbiota. However, this high degree of specificity is also a disadvantage when there is an urgent need for treatment and the source of infection is unclear. This could be addressed through the application of multi-phage cocktails (Nilsson, 2014).

Due to the self-replicative nature of phages, phage therapeutics can be used as active medicines, in which a single phage dose in a relatively high concentration of bacteria could sustain the propagation of the phage. This self-sustaining capability may also reduce the impact of immune clearance and the poor diffusion of phages (Brüssow, 2013 & Nilsson, 2014).

Resistance to phages is neither global nor transferable when compared to antibiotics, i.e. bacterial phage resistance is generally confined to a single phage. As for the clustered regularly interspaced short palindromic repeats (CRISPR) and their associated genes linked

to a mechanism of acquired resistance against bacteriophages, where bacteria can integrate short stretches of phage-derived sequences (spacers) within CRISPR loci to become phage resistant (Deveau et al., 2008).

Barrangou & Horvath (2009) have stated a few points on how phages can adapt to CRISPR-encoded resistance; CRISPR-imposed selective pressure can lead to specific mutations in the proto-spacer sequence to which a single nucleotide change which allows a phage to circumvent CRISPR-encoded immunity by altering targeted sequences via deletions. Phages may also circumvent the CRISPR when they develop mutations in the direct vicinity of the protospacer. Landsberger et al., (2018) studied on phages encoding for anti-CRISPR (Acr) genes, which antagonise bacterial CRISPR-Cas immune systems by binding components of its machinery. Bacteria with CRISPR-Cas resistance is still partially immune to Acr-encoding phage. Therefore, Acr-phages often need to cooperate in order to overcome CRISPR resistance, with a first phage blocking the host CRISPR-Cas immune system to allow a second Acr-phage to successfully replicate. Even though bacteria eventually develop resistance to phage infection, the isolation of a new phage is relatively fast and cheap compared to the discovery of a new antibiotic (Sulakvelidze et al., 2001).

Although phages are commonly believed to be non-immunogenic and non-toxic, they, however, can potentially be toxic to humans. For example, β -phage infecting *Corynebacterium diphtheriae* secreting diphtheria toxin, causing diphtheria (Holmes, 2000). The presence of bacterial endotoxins that are present as part of the phage preparation, or that are released as part of lytic replication, may result in septic shock (Loc-Carrillo & Abedon, 2011). Thus, it is crucial that all phages are fully characterised prior to use and minimising the endotoxin content. Nevertheless, bacterial endotoxins can be removed synthetically from

aqueous bacteriophage lysates while retaining the infectivity of the bacteriophages (Szermer-Olearnik & Boratynski, 2015).

Phages primarily use one of two main life cycles, the lytic or lysogenic cycle. Despite the potential for using temperate phages as gene delivery vehicles, temperate phages are not suitable for phage therapy and best avoided. This is due to the potential for the transfer of virulence elements such as toxins or the ability of temperate phages to mediate resistance to other phages from the same immunity group (Loc-Carrillo & Abedon, 2011). As mentioned in the previous paragraph, the toxinogenic phenotype of *C. diphtheriae* is determined by temperate corynephages (β -phage) whose genomes carry the *tox* gene that encodes diphtheria toxin (Zajdowicz & Holmes, 2016). Therefore, obligately lytic bacteriophages are highly desirable for therapeutic uses. Because they result in the rapid killing of their target host cell, bacteriophage numbers increase rapidly, and transduction is relatively rare. Self-replicating effect of obligate lytic phages is a much-touted advantage, as it implies that phages are self-sustaining as long as host bacteria are present (Mirzaei, 2016).

1.6.4 Potentials, Safety and Current Clinical Trials of Therapeutic Bacteriophages

In the following decades, only a few institutes continued to research and use phages as a treatment method, most notably the Eliava Institute in Georgia and the Wroclaw Institute of Experimental Therapy in Poland. These institutes are actively involved in the development and administration of phages to patients (Eriksson, 2015).

There are several commercial entities currently working within this field, with phage products for food processing (Intralytix Inc., Microcos BV) and human phage therapy product investigations (Ampliphi Biosciences corp., Novolytics ltd.). Clinical trials for phage therapy are underway; for example, in 2009 a successful double-blind, placebo and

randomised phase II clinical trial was performed using a phage cocktail consisting of six different phages. This study investigated the safety and efficacy of using phages as a treatment method for multi-resistant *Pseudomonas aeruginosa* in chronic otitis patients (Wright et al., 2009). The treated group showed a 76 % decrease in bacterial counts, while the placebo group showed a 9 % increase in bacterial cell density in the place of infection.

A phage therapy study funded by Nestlé ran extensive trials of phage cocktails in Bangladesh (Sarker et al., 2012). Nestlé's main bacterium of interest is *E. coli*, which contaminates many sources of drinking water, causing severe dehydration due to diarrhoea in local populations. The safety trials were performed in adults, but the project was terminated in 2013. Eriksson (2015) briefly mentioned on the PhagoBurn project launched in 2013, received funding from the European Union 7th framework program to evaluate phage therapy treatment of *E. coli* and *P. aeruginosa* infections in burn wound infections in clinical trials. Through the PhagoBurn project, significant advancements were achieved regarding the regulatory framework of phage therapy and led to the world first GMP-like production of bacteriophage drug products.

A controlled phase I/II clinical trial to treat chronic otitis, infected by drug-resistant *P. aeruginosa*, is one such example of a successful phage application. In the study, 24 patients with chronic otitis were divided into two groups and treated with a single dose (presumably via topical application) of a phage preparation 6×10^5 phages or a placebo. Forty-two days after treatment, the phage-treated group showed a significant improvement in their clinical indicators compared to those given the placebo (Wright et al., 2009).

A phase I safety trial of a phage cocktail against three infecting pathogens (*E. coli*, *P. aeruginosa* and *S. aureus*) of chronic venous ulcers showed no side effect on patients.

The trial was conducted with 39 patients who were treated topically for 12 weeks with a phage cocktail while the control group received saline. Patients were monitored for adverse effects until week 24. No significant difference between the treatment and control groups were observed for either the rate of wound healing or for adverse side effects (Rhoads et al., 2009). A very recent work by Robert et al., (2017) have reported on a positive outcome using a customised bacteriophage-based therapeutic treatment for a diabetic patient with necrotising pancreatitis complicated by MDR *A. baumannii* infection. The isolated lytic bacteriophages were administered into the patient by intravenous and percutaneous method into the abscess cavities.

1.6.5 Isolated Bacteriophages Infecting *Klebsiella pneumoniae*

As an obligate parasite, phages are dependent upon the survival of their host population. Thus, the availability of hosts is important in determining the environmental robustness of phages. Phages must be able to manipulate the gene expression of their bacterial host cells to prevent superinfection by other phages as well as to provide a competitiveness nature for their hosts to thrive in the ecosystem (Chaturongakul & Ounjai, 2014).

Phage development and infection processes depend not only on the physicochemical characteristics of their genome-encoded functions but also on the host cell intracellular resources and thus, subsequently dependent on their host physiological state. Different stages of phage growth, including the attachment of the phage particle to its host, the penetration of phage DNA into its host, and the synthesis of the phage components, have been found to be sensitive to the physiological state of the host, in turn, is modulated by its growth medium, temperature, oxygen tension, and pretreatment using chemical agents. These studies have

shown that the faster that the host grows at the time of phage infection, the faster that the phage will grow, corresponding to a shorter latent time, faster progeny production rate, and larger burst size (You et al., 2002).

Recent reports highlighted the successful treatment of *Klebsiella pneumoniae*–induced burn wound infection in mice with the *Klebsiella*-specific phage Kpn5 incorporated in a 3 % hydroxypropyl-methylcellulose hydrogel (Kumari et al., 2010). On bacterial challenge, mice were treated once with low and high doses of phage hydrogel (MOI of 1 or 100, respectively). Significant levels of protection against invasive *K. pneumoniae* infection were achieved only by high titers of phage. These results indicate that the dosage of phage is important for effective phage therapy.

In another set of experiment, Chadha et al., (2016) evaluated the therapeutic efficacy of five mono-phages namely Kpn1, Kpn2, Kpn3, Kpn4 and Kpn5 in comparison to phage cocktail in resolving the course of *K. pneumoniae* B5055 induced-burn wound infection in mice. Both mono-phages therapy and phage cocktail exhibited efficacy in resolving the course of infections. However, phage cocktail showed maximum protection against burn wound infection by *K. pneumoniae* B5055.

In addition, there were other studies and experiments conducted by multiple researchers namely, Wu et al., (2007); Drulis-Kawa et al., (2011); Karumidze et al., (2013) and Hoyles et al., (2015) in isolating bacteriophages from different samples, such as sewage and caecal effluent to infect *K. pneumoniae* strains that exhibit different characteristics. It was hypothesised that lytic bacteriophages could be isolated from local sewage water in Sarawak.

1.7 Study Objectives

The development of novel therapeutics to treat drug-resistant infections, especially those caused by six nosocomial pathogens (ESKAPE) mentioned in Section 1.3 is called-for. As the isolation of a new phage is relatively fast and cheap compared to the discovery of a new antibiotic since phages are readily available and easily isolated from the environment, it can be a potential replacement for antibiotics as antimicrobial agents.

Therefore, the overall objectives of this study were to isolate and characterise lytic bacteriophages with therapeutic potential against clinical- and community-acquired *K. pneumoniae* strains from sewage samples. The specific aims of this research are to:

- i. To isolate lytic bacteriophages from sewage samples infecting *Klebsiella pneumoniae*.
- ii. To characterise the isolated lytic bacteriophages infecting *Klebsiella pneumoniae*.
- iii. To evaluate bacteriophages efficacy in cocktail and synergy reaction with antibiotic against antibiotic resistant *Klebsiella pneumoniae*.

It is hoped that this study will be able to contribute to future phage therapy studies and applications.

CHAPTER 2

MATERIALS AND METHODS

2.1 Culture Medium Sterilisation

2.1.1 Filter Sterilisation

Culture medium was sterilised by passing through a 0.22 µm PES syringe filter (Sartorius Minisart®).

2.1.2 Heat Sterilisation

Culture medium was subject to moist-heat sterilisation by autoclaving at 121 °C for 15 mins in a top-loading autoclave (TOMY Seiko).

2.2 Culture Media and Reagents Preparation

2.2.1 Preparation of Media Broth – Luria-Bertani (LB) Broth

Single strength of LB broth was prepared by weighing 10 g of tryptone powder (Oxoid, UK), 5 g of yeast extract (Oxoid, UK) and 10 g of sodium chloride, NaCl (ThermoFisher, U.S.A.) added into a 1 L media bottle (Duran®, Germany) and topped up to 1 L with distilled water. The mixture was homogenised, and heat sterilised according to Section 2.1.2. LB double strength (2x) medium preparation was done by doubling the ingredients quantity to 1 L of distilled water and quintuple quantity respectively for 5x LB strength medium.

2.2.2 Preparation of Media Agar – Luria-Bertani (LB) Agar

LB agar was prepared by weighing 10 g of tryptone powder (Oxoid, UK), 5 g of yeast extract (Oxoid, UK), 10 g of sodium chloride, NaCl (ThermoFisher, U.S.A.) and 15 g

of agar bacteriological powder (Oxoid, UK), added into a 1 L media bottle (Duran®, Germany) and topped up to 1 L with distilled water. The mixture was mixed well and autoclaved according to Section 2.1.2. The autoclaved media was then poured into a petri dish (Favorit®, Malaysia).

2.2.3 Preparation of Media Agar – Mueller-Hinton (MH) Agar

Mueller-Hinton agar was prepared by adding 38 g of Mueller-Hinton agar powder (Oxoid, UK) in 1 L distilled water in a 1 L media bottle (Duran, Germany) and was mixed well. The mixture was then autoclaved according to Section 2.1.2. The autoclaved media was then poured into a Petri dish (Favorit®, Malaysia).

2.2.4 Preparation of Glycerol Solution (50 % v/v)

A 50 % glycerol solution was prepared by adding 50 mL of 100 % glycerol (ThermoFisher, U.S.A.) in 50 mL of distilled water. The mixture is then stirred homogenously and stored in 4 °C refrigerator.

2.2.5 Preparation of SM Buffer

SM buffer was prepared by adding 5.8 g NaCl, 1.2 g MgSO₄ and 50 mL 1M Tris-HCl, pH 7.5 in 1 L distilled water. The medium was filter-sterilised according to Section 2.1.1 before use, and store at room temperature. SM buffer is used for phage dilution and storage.

2.2.6 Preparation of Phosphate Buffered Saline (PBS) Buffer

Phosphate buffered saline was prepared by dissolving 1 tablet of PBS (Oxoid, UK) in 100 mL of distilled water and filter-sterilised according to Section 2.1.1.

2.2.7 Preparation of 1X Tris-Acetate EDTA (TAE) Buffer

One hundred mL of 10X Tris-Acetate-EDTA (TAE) buffer, pH 8.0, Ultra-Pure Grade is added to 1 L distilled water and filter-sterilised according to Section 2.1.1.

2.2.8 Preparation of 0.1M Calcium Chloride (CaCl₂) Solution

Soluble 14.7 g of Calcium Chloride dihydrate, CaCl₂.2H₂O (Fisher Scientific) is added to 1 L distilled water and filter-sterilised according to Section 2.1.1.

2.3 Antibiotic used for Antibiotic Sensitivity Testing (AST)

Table 2.1: List of Antimicrobial Agents used against *Klebsiella pneumoniae*

Antimicrobial Agents	Concentration (µg)
β-lactams	
Ampicillin	20
Amoxicillin	-
Sulbactam Ampicillin	-
Piperacillin-tazobactam	-
Cefuroxime	-
Ceftazidime	30
Ceftriaxone	-
Cefepime	-
Imipenem	10
Meropenem	10
Non-β-lactams	
Amikacin	30
Ciprofloxacin	5
Gentamicin	10
Trimethoprim/sulfamethoxazole	-
Vancomycin	10

AST of *Klebsiella pneumoniae* clinical strains was assessed by Borneo Medical Center (BMC) according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Only selected concentration of antimicrobial agents was reported as shown in

Table 2.1. AST of community-acquired *K. pneumoniae* strains was performed according to Section 2.7.

2.4 Bacterial Strains

In this study, a total of twenty-one *Klebsiella* spp. strains, as shown in Table 2.2, were obtained from Borneo Medical Centre (BMC) Diagnostic Lab and student swab samples of University Malaysia Sarawak (UNIMAS). A diverse group of *Klebsiella* spp. isolates were acquired between the year 2016 and 2017 to be used as tools for phage isolation and characterisation.

The collection of specimens from BMC and UNIMAS students were approved by the UNIMAS Medical Ethics Committee with the reference number of UNIMAS/NC-21.02/03-02 Jld.2 (79) and UNIMAS/NC-21.02/03-02 (79) respectively (Appendix I).

Table 2.2: *Klebsiella* spp. isolates used throughout the experiment

Strain No.	Assigned Name	Lab Sequence No.	Category
1	CRKP1	4090	CLINICAL STRAINS
2	ESKP2	4100-1	
3	ESKP3	3492	
4	ESKP4	1692	
5	KP5	1885	
6	KP6	1713	
7	KP7	1804	
8	KP8	1779	
9	KP9	1829	
10	KP10	3689	
11	KP11	3539	
12	KP12	1553	
13	KP13	2056	
14	KP14	5296	

Table 2.2 continued

15	KP15	41205A	COMMUNITY ACQUIRED
16	KP16	38358.2	
17	KP17	42788.2	
18	KA1	32282a	
19	KA2	38252.1	
20	KO1	32044	
21	ATCC KP	ATCC 35657	LAB STRAIN

*The assigned name corresponds to the lab sequence number shown in Table 2.2 and will be referred to as such throughout this thesis.

In addition, a reference strain of *K. pneumoniae* ATCC KP from the American Type Culture Collection (ATCC) was also used in this study as a positive control. All bacteria cultures were stored in Luria-Bertani broth (LB) containing glycerol (v/v 15 %) at -20 °C for future analysis. Throughout each experiment, *Klebsiella* spp. strains were inoculated in Luria-Bertani (LB) media – broth or agar and were incubated overnight at 37 °C.

2.5 Preparation of *Klebsiella* spp. Log-phase Culture Broth

An isolated colony of *Klebsiella pneumoniae* ATCC KP was inoculated into 15 mL LB broth and incubated at 37 °C. 100 µL of the overnight culture was transferred into 10 mL of fresh 1X LB broth. The mixture was shaken to approximately 3-5 h at 37 °C in the incubator shaker until it turns slightly cloudy. One mL of the suspension was then transferred to a clean cuvette and placed in UV-VIS spectrophotometer to determine the optical density (OD). The bacterial suspension is in its log-phase once the OD reaches OD₆₀₀ = 0.3. This procedure was applied to all *Klebsiella* spp. clinical strains.

2.6 Verification of *Klebsiella pneumoniae* Genomic DNA

2.6.1 Wizard® Genomic DNA Purification Kit by Promega

Bacterial DNA genome is purified according to the DNA purification kit protocol. Firstly, 1 mL of overnight culture is centrifuged for 2 mins at 13,000-16,000 x g to obtain a cell pellet. The supernatant is discarded. To lyse the cell, 600 µL of Nuclei Lysis Solution is added and pipetted gently to mix. The mixture is incubated for 5 mins at 80 °C then cooled to room temperature. 3 µL of RNase Solution is added and incubated at 37 °C for 15-60 mins. To precipitate the bacterial protein, 200 µL of Protein Precipitation Solution is added and mixed by vortex. The solution is then incubated on ice for 5 mins and centrifuge at 13,000-16,000 x g for 3 mins. For DNA precipitation and rehydration, the supernatant is transferred to a new clean tube containing 600 µL of room temperature isopropanol and centrifuged for 2 mins at 13,000-16,000 x g. The supernatant is decanted. 600 µL of room temperature 70 % ethanol is added to the pellet followed by 2 mins centrifuge at 13,000 – 16,000 x g. The leftover ethanol is aspirated, and the DNA pellet is air-dried for 10-15 mins. Lastly, the pellet is rehydrated in 100 µL of Rehydration Solution for 1 h at 65 °C. The isolated genomic DNA is stored at 4 °C for further analysis.

2.6.2 Bacterial 16S rRNA PCR primers

A universal primer pair used in *Klebsiella pneumoniae* 16S rRNA sequencing (Lean et al., 2014) as stated:

Forward primer: [27F] 5'-AGAGTTTGATCMTGGCTCAG-3'

Reverse primer: [152R] 5'-AGGAGGTGWTCCARCC- 3'

Table 2.3: Components of *Klebsiella pneumoniae* 16S rRNA PCR mixture in 50 μ L reaction

Reagent	Volume (μ L)	Final Concentration
1. 10x reaction buffer	5.00	1x
2. dNTPs (10 μ M each)	1.00	200 μ M
3. MgCl ₂ (25 μ M)	3.00	1.5 μ M
4. PCR forward primer (100 μ M)	0.50	1 μ M
5. PCR reverse primer (100 μ M)	0.50	1 μ M
6. <i>Taq</i> DNA polymerase (5/500 u)	0.25	1.25u/50 μ L
7. DNA Template	5.00	<0.5 μ g/50 μ L ~100 ng
8. Ultra-pure H ₂ O	34.75	-
Total	50 μL	

2.6.3 Bacterial 16S rRNA PCR Condition

Fifty microlitres (50 μ L) of PCR mixture (refer to Table 2.3) with the final volume of the template DNA, 1-2 ng / μ L and final primers concentrations of 1 pmol / μ L were amplified. The PCR amplification was performed based on Lean et al., (2014) using a Veriti 96 Well Thermal Cycler (Applied Biosystems, U.S.A) under the following temperature profile: 95 °C for 3 mins, 34 cycles consisting of 95 °C for 50 s (denaturation), 60 °C for 50 s (annealing), 72 °C for 1 min (extension), and final elongation of the amplified DNA at 72 °C for 90 s.

2.6.4 Agarose Gel Electrophoresis

The PCR products were separated by 1.0 % agarose gel electrophoresis stained with Ethidium Bromide in 1X Tris-acetate-EDTA (TAE) buffer at 100 V for 60 mins. 1 kb DNA Ladder (Promega) was used as a molecular sizes standard.

2.6.5 Bacterial 16S rRNA Sequencing

The PCR products were sent to Apical Scientific Sdn. Bhd. (private laboratory) using the ABI Big Dye sequencing kit and ABI prism sequencer for the 16S rRNA partial sequencing to verify the bacterial strains used in this study. The sequence data was then submitted for NCBI BLAST® analysis to identify the sequence based on its similarity with the other sequences available in the GenBank database.

2.7 Antibiotic Sensitivity Testing on *Klebsiella pneumoniae* Community-acquired Isolates

Susceptibility testing was performed for community-acquired *Klebsiella pneumoniae* isolates by the standard Kirby-Bauer disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. ATCC KP was used as a control strain and was run simultaneously with the test organisms. *K. pneumoniae* strains were cultured in Luria-Bertani broth for 18-24 h. Each culture of *K. pneumoniae* strain was swabbed on the surface of the LB agar plate. After completely swabbing the plate, the agar plate was turned 90 degrees and the swabbing process was repeated. The agar plate was then left to air dry and different antibiotic disks were put onto the surface of the agar using aseptic technique. The agar plates were incubated overnight at 37 °C. The next day, the agar plates were observed, and the diameters of the inhibition zone were measured.

2.8 Housekeeping Genes Sequencing of *Klebsiella pneumoniae*

Bacterial isolates (n= 21) were selected for housekeeping genes sequencing and were performed according to the protocol described by Diancourt et al., (2005). However, only three selected housekeeping genes were used in this study which are: gapA (glyceraldehyde 3-phosphate dehydrogenase, target sequence 450 bp), rpoB (beta-subunit of RNA polymerase, target sequence 501 bp) and tonB (periplasmic energy transducer, target

sequence 414 bp) with the following modification: universal sequences were added to upstream of each forward primers (as to Section 2.8.1).

2.8.1 Housekeeping PCR primers (Diancourt et. al., 2005)

Universal sequences were added upstream of each primer (**Forward**) 5'-GTT TTC CCAGTCACGACGTTGTA-3' and (**Reverse**) 5'-TTGTGAGCG GAT AACAAAT TTC-3.

Table 2.4: Universal sequencing primers for *Klebsiella pneumoniae* housekeeping genes

Housekeeping Gene	Primer	Primer Sequences
rpoB	Forward: Vic3oF	5'- GTT TTC CCA GTC ACG ACG TTG TA GGC GAA ATG GCW GAG AAC CA-3'
	Reverse: Vic2oR	5'- TTG TGA GCG GAT AAC AAT TTC GAG TCT TCG AAG TTG TAA CC-3'
gapA	Forward: 173oF	5'- GTT TTC CCA GTC ACG ACG TTG TA TGA AAT ATG ACT CCA CTC ACG G-3'
	Reverse: 181oR	5'- TTG TGA GCG GAT AAC AAT TTC CTT CAG AAG CGG CTT TGA TGG CTT-3'
tonB	Forward: tonB1F	5'- GTT TTC CCA GTC ACG ACG TTG TA CTT TAT ACC TCG GTA CAT CAG GTT-3'
	Reverse: tonB2R	5'- TTG TGA GCG GAT AAC AAT TTC ATT CGC CGG CTG RGC RGA GAG-3'

Note: **Red** font indicates on the respective *Klebsiella pneumoniae* housekeeping gene primers added to the consensus primers mentioned above in Section 2.9.1

Table 2.5: Components of *Klebsiella pneumoniae* housekeeping genes PCR mixture in 25 μ L reaction

Reagent	Volume (μ L)	Final Concentration
1. 10x reaction buffer	5.00	1x
2. dNTPs (10 μ M each)	1.00	200 μ M
3. MgCl ₂ (25 μ M)	3.00	1.5 μ M
4. PCR forward primer (10 μ M)	1.00	1 μ M
5. PCR reverse primer (10 μ M)	1.00	1 μ M
6. <i>Taq</i> DNA polymerase (5/500u)	0.25	1.25 u / 50 μ L
7. DNA Template	5.00	<0.5 μ g / 50 μ L ~100 ng
8. Ultra-pure H ₂ O	8.75	-
Total	25 μL	

Table 2.6: PCR cycle condition for 25 μ L of *Klebsiella pneumoniae* housekeeping genes

Condition	Temperature ($^{\circ}$ C)	Time
Initial denaturation	94	2 mins
Denaturation (35 cycles)	94	30 s
Annealing	50	1 min
Extension	72	30 s
Final Extension	72	5 mins
Hold	4	∞

PCR amplifications were performed based on Table 2.5 and Table 2.6 at an annealing temperature of 50 $^{\circ}$ C for all genes, and the sequences were obtained using two universal sequencing primers mentioned in Section 2.8.1. Further details are available on the *K. pneumoniae* MLST website (<https://bigsd.b.pasteur.fr/>).

2.9 Bacteriophage Isolation, Purification, and Titration

2.9.1 Bacteriophages Sample Collection

The sewage sample was collected from a student residential college sewage tank in University Malaysia Sarawak (UNIMAS, Malaysia) as shown in Figure 2.1. Sampling was

done during a clear and sunny day to prevent having diluted bacteriophage samples. 500 mL sterile bottles were used to collect the sewage sample and kept in 4 °C refrigerator for further analysis.

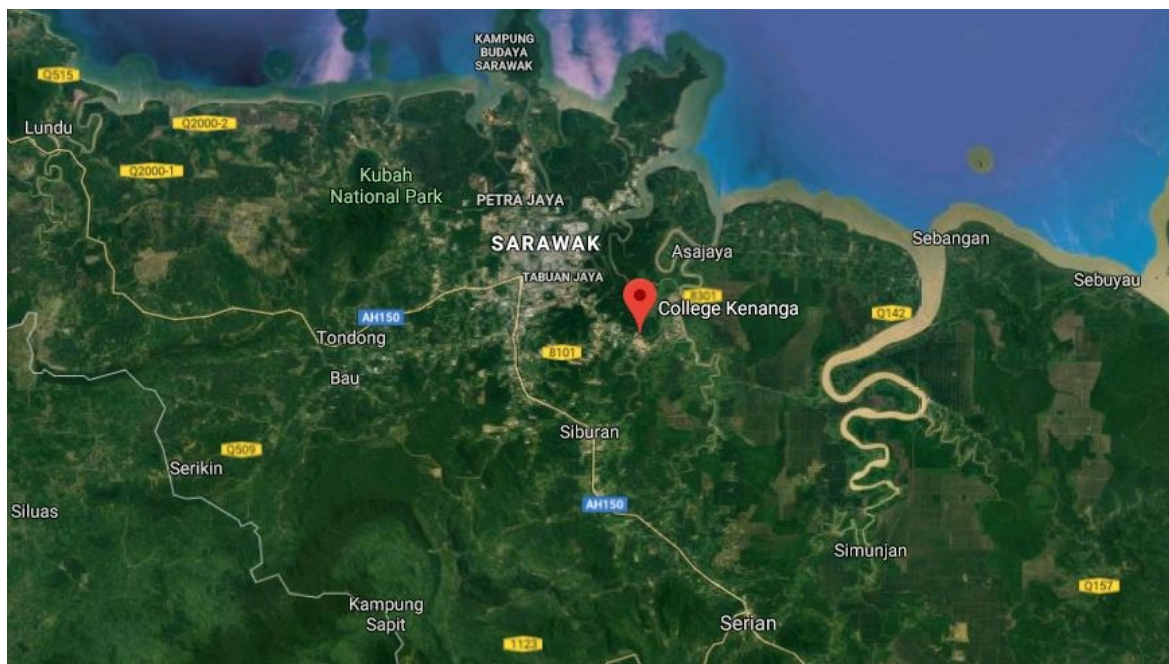


Figure 2.1: Location of sampling site in Sarawak with GPS coordinates; Latitude: 1° 27' 34.79" N and Longitude: 110° 26' 23.39" E indicated with red tag.

2.9.2 Bacteriophages Enrichment

Concentrated bacteriophage lysate is prepared by culturing 100 µL of overnight *Klebsiella pneumoniae* culture into 30 mL of 5X LB liquid medium with raw wastewater sample added to a final volume of 50 mL. The mixture was incubated at 37 °C with shaking overnight in an incubator shaker. The overnight enriched bacteriophage culture was then filtered according to Section 2.1.1.

2.9.3 Bacteriophages Serial Dilution

Ninety microliters of SM Buffer supplemented with 1 mM CaCl₂ were aliquoted into microcentrifuge tubes for each dilution. The tubes were labelled with the dilution number of 10⁻¹ to 10⁻⁹. Ten µL of the undiluted phage lysate (as to Section 2.9.2) was added to the first dilution tube labelled 10⁻¹. The tube is then vortexed briefly to mix. 10 µL from tube 10⁻¹ was removed with a new pipette tip and added to the 10⁻² tube. This process was repeated until the required number of dilutions has been made.

2.9.4 Plaque Assay – Double-layer Agar Overlay Technique

Diluted bacteriophage culture (as to Section 2.9.3) were mixed with 300 µL of overnight *Klebsiella pneumoniae* in a pre-warmed sterile bijou bottle and incubated for 5 mins at 37 °C. The mixture was then mixed with 3 mL of molten (45 °C) LB top agar and overlaid onto 1.5 % LB agar plate. They were incubated at 37 °C overnight.

Phage plaques were picked from the agar plate and single phage plaque was purified on host strain by the standard procedure (Sambrook et. al., 1989). The purification procedure was repeated three times to ensure pure phage plaque was obtained. The purified bacteriophages were stored in the phage SM buffer at 4 °C.

2.9.5 Identifying and Verifying Putative Plaques (Spot Assay)

Phage activity was detected by spot assay (Chang et al., 2005). Overnight cultures prepared in LB medium were then sub-cultured by addition of 100 µL to 10 mL LB broth and grown at 37 °C for 90 mins. 100 µL of sub-culture was inoculated into 3 mL of molten LB top agar and overlaid onto LB agar plates. Each overlay was left to solidify for 15 min. All phage lysates were diluted in 10-fold increments (to Section 2.9.3) and 10 µL of each dilution was spotted onto the bacterial overlay, dried, then incubated at 37 °C overnight. As

a control, each bacterial strain was mock-infected with sterile SM buffer. Results were analysed based on detection of any lysis (clear plaque formation) and further dilutions were checked for single plaques to ensure phage lysis rather than bacteriocin induced lysis. All spot tests were repeated in triplicate to confirm results.

2.9.6 Bacteriophage Titration

Bacteriophage titer is expressed as plaque-forming units (PFU) per mL using the double-layer agar method also known as Full Plate Titer. A series of dilution was performed (as to Section 2.9.3) in sterile SM buffer for plaque assay (as to Section 2.9.4). Only plates with 30-300 plaques per plate were selected. The titer of the bacteriophage lysate by using the following calculation:

$\frac{\text{PFU}}{\text{ml}} = \frac{\text{Number of plaques}}{d \times V}$ where, d = dilution V = volume of diluted virus added to the plate	Equation 2.1
--	--------------

2.10 Bacteriophages Characterisation

2.10.1 Phage Host Range Analysis

Individual phages were tested for their ability to lyse *Klebsiella* spp. by spot assay (as to Section 2.9.5). These assays were performed on a range of different bacteria that includes carbapenemase-resistant *K. pneumoniae* (KPC), extended-spectrum β -lactamase (ESBL) *K. pneumoniae*, *K. aerogenes*, and *K. oxytoca*. Each *Klebsiella* sp. strain was grown individually to log phase (as to Section 2.5), inoculated into molten LB top agar and overlaid onto LB agar plates. Then phage lysate was spotted onto the bacterial overlay as described

previously in the spot assay method (as to Section 2.9.5). The co-culture was incubated overnight at 37 °C and individual phage titers were determined based on Equation 2.1.

2.10.2 Step-by-Step (SBS) Phage Isolation Method

Step-by-Step (SBS) approached by Gu et al., (2012) was applied to isolate phages using wild-type bacteria and consecutively phage-resistant variants as hosts. Simply, the first and the second phages were isolated using wild-type bacteria and the first phage-resistant variant (resistant to the first phage) as hosts, respectively. The next variant, resistant to the second phages, was used to isolate the third phage. The process of phage isolation, according to Section 2.9.3 till Section 2.9.5 was stopped until the last phage-resistant bacteria were sensitive toward isolated phage.

2.10.3 Single-Step Growth Curve

To determine the different phases of phage infection, a single-step growth curve of phages propagated on *K. pneumoniae* strains was performed according to Delbruck (1940). A volume of 0.1 mL of the phage lysate was added to 0.9 mL of host culture in a conical tube labelled “adsorption tube” and were mixed well. The exact time was recorded, and the tubes were incubated in 37 °C water bath. Two large test tubes containing 9.9 mL of LB broth were labelled 10^{-2} and 10^{-4} were then placed at 37 °C.

At precisely 10 mins after starting the incubation of the Adsorption Tube, 0.1 mL of adsorption mixture were transferred to the tube marked 10^{-2} and was vortexed to mix. Another sterile pipet was used to transfer 0.1 mL from tube 10^{-2} to tube 10^{-4} and was vortexed to mix. At exactly 20 minutes into the experiment, 0.1 mL from the 10^{-4} tube was added together with 0.1 mL of the host culture to a soft agar tube and thoroughly mixed by rolling the tube using hand palms.

The soft agar was poured onto an agar plate labelled "20 mins" and the plate was rotated to evenly spread the soft agar across the surface of the entire plate. After the agar hardened, the plate was inverted and incubated at 37 °C overnight. The procedures were repeated at precisely 25, 30, 40, 50, 60, 70 and 80 mins into the experiment.

2.10.4 Multiplicity of Infection (MOI)

To determine the optimal multiplicity of infection (MOI) of phages, the method performed by Mishra et al., (2014) was applied with mid-log phase host cells (as to Figure 3.3 in Section 3.4) were infected with the phages at 4 different ratios (0.01, 0.1, 1, and 10; pfu / cfu). After incubation for 4 h at 37 °C, the phage lysate was centrifuged at 1000 × g for 3 min. The supernatant was filtered according to Section 2.1.1 and assayed to determine the phage titer (as to Section 2.9.6). The MOI resulting in the highest phage titer within 4 h was considered as the optimal MOI and was used for the bulk production of the phages, as well as subsequent experiments.

2.10.5 High Titer Stock of Purified Bacteriophages

Three-hundred microliter of *K. pneumoniae* overnight culture (KP15, KP17, CRKP1 and ATCC KP) was inoculated into 10 mL of pre-warmed (37 °C) 5x LB, spiked with 0.1 M CaCl₂. The bacterial culture was incubated for 1 h at 37 °C in an incubator shaker. Hundred µL phage was added to the culture and incubated overnight at 37 °C with agitation. The bacteria-phage suspension was then filtered according to Section 2.1.1.

2.10.6 Bacteriophage DNA Extraction

The bacteriophages genomic DNA were extracted and purified using Roche High Pure Viral Nucleic Acid Kit (Roche Diagnostics GmbH, Germany) according to the supplied protocol with slight modification.

Two hundred microliters of the binding buffer were added to 200 μ L of bacteriophage lysate (as to Section 2.10.5) supplemented with 55 μ L of pre-mixed poly(A) and Proteinase K. Sample was immediately mixed and incubated for 10 mins at 72 °C and afterwards, the 100 μ L binding buffer was added to the sample. The sample was then pipetted in the upper reservoir of the High Pure Filter Tube and centrifuged for 1 min at 8000 x g. Next, 500 μ L Inhibitor Removal Buffer was added and next, centrifuged at the same condition. The sample was washed twice with 450 μ L Wash Buffer followed by centrifugation after each wash. After the second wash, additional centrifugation was performed for 10 s at the maximum speed (13,000 x g) to completely remove the washing buffer. Lastly, 30 μ L of Elution Buffer was added to the sample and incubated at room temperature for 3 mins. After 3 mins, the sample was again centrifuged for 1 min at 8,000 x g. The product of centrifugation was the purified bacteriophages genomic DNA which were then kept at 4 °C for later analysis.

2.10.7 Characterisation of Genes Encoding Bacteriophage Proteins

PCR method was used to amplify the isolated bacteriophages proteins as shown in Table 2.8 using The Veriti® 96-Well Thermal Cycler (Applied Biosystems, U.S.A.). There were four pairs of primers used to target the different genes according to Table 2.7. The genes targeted were lysin gene, viral capsid assembly protein gene (g20), major capsid protein gene (g23) and tail fiber protein gene (T1). The oligonucleotide primers used in this study were synthesised by Integrated DNA Technologies (Singapore).

Table 2.7: Specific primers used for Phage Structural Protein analysis

Bacteriophage Gene	Primer Sequence
Lysin Gene (Mishra et al., 2014)	Forward: 5'-ATGGCTAAGACTCAAGCAGAA-3' Reverse: 5' - ATTTACCCGTGTGCCAAG-3'
Viral Capsid Assembly Protein Gene (g20) (Jing et al., 2014)	Forward (CPS1): 5'-GTA GWA TTT TCT ACA TTG AYG TTG G-3' Reverse (CPS8): 5'-AAA TAY TTD CCA ACA WAT GGA-3'
Major Capsid Protein Gene (g23) (Short et al., 2010)	Forward (MZIA1bis): 5'-GAT ATT TGI GGI GTT CAG CCI ATG A-3' Reverse (MZIA6): 5'-CGC GGT TGA TTT CCA GCA TGA TTT C-3'
Tail Fibre Protein Gene (T1) (Hee, 2009)	Forward: 5'-GYG AYC AGA TGG TTC C-3' Reverse: 5'-CAA TRT CYT CYT ART TG-3'

Table 2.8: Components of *Klebsiella* phage structural protein PCR mixture in 30 µL reaction

Reagent	Volume (µL)
1. Ultra-Pure H ₂ O	12.5
2. Forward Primer (20 pmol)	1
3. Reverse Primer (20 pmol)	1
4. 5x Green GoTaq® Flexi Buffer (Promega, WI, U.S.A.)	5
5. 25 mM MgCl ₂	2
6. 10 mM dNTP mix (Promega, WI, U.S.A.)	0.5
7. GoTaq® DNA Polymerase (Promega, WI, U.S.A.)	3
8. DNA template	5
Total	30 µL

Phages were identified firstly through Lysin (Lys) gene amplification using method conducted by Mishra et al., (2014).

Table 2.9: PCR condition for Lysin gene (Lys)

Condition	Temperature (°C)	Time
Initial denaturation	94	5 mins
Denaturation (30 cycles)	94	60 s
Annealing	50	60 s
Extension	72	60 s
Final Extension	72	7 mins
Hold	4	∞

Secondly, Viral Capsid Assembly Protein gene (g20) was amplified through method applied by Jing et al., (2014) with slight modification.

Table 2.10: PCR condition for Viral Capsid Assembly protein gene (g20)

Condition	Temperature (°C)	Time
Initial denaturation	94	3 mins
Denaturation (35 cycles)	94	15 s
Annealing	45	15 s
Extension	73	1 min
Final Extension	73	4 mins
Hold	4	∞

Next, Major Capsid Protein gene (g23) was amplified using protocol conducted by Short et al., (2010).

Table 2.11: PCR condition for Major Capsid Protein gene (g23)

Condition	Temperature (°C)	Time
Initial denaturation	95	5 mins
Denaturation (30 cycles)	95	30 s
Annealing	57	30 s
Extension	72	60 s
Final Extension	72	5 mins
Hold	4	∞

Lastly, the method of amplifying Tail Fiber Protein gene T1 and primers used by Hee (2009) was applied.

Table 2.12: PCR condition for Tail Fiber protein gene (T1)

Condition	Temperature (°C)	Time
Initial denaturation	95	5 mins
Denaturation (40 cycles)	95	30 s
Annealing	40	30 s
Extension	72	30 s
Final Extension	72	10 mins
Hold	4	∞

The closest relatives of the respective lysin, g20, g23, and tail fiber protein gene clones were examined using the Basic Local Alignment Search Tool (BLAST) search program at the National Center for Biotechnology Information (NCBI) website (<http://www.ncbi.nlm.nih.gov/>) at the amino acid level.

2.11 Phage Cocktail and Phage Antibiotic Synergistic against Antibiotic-Resistant *Klebsiella pneumoniae*

2.11.1 Phage Cocktail

The cocktail comprised an equal amount of each phage. The isolated phages with broad host range as shown in Table 3.6 were combined based on the volume of one to one ratio into a cocktail and tested for their ability to lyse bacteria cultures of *Klebsiella pneumoniae*.

The bacteria strains were grown to log-phase growth (as to Section 2.6) in 100 mL flasks of nutrient broth. The flasks were inoculated with individual phages (determination of optimal MOI according to Section 2.10.4) and incubated at 37 °C with shaking. Bacterial growth at OD₆₀₀ was then analyzed by spectrophotometer at 0 h, 1 h, 2 h, 3 h, and 24 h to determine the viability of the phages over time.

2.11.2 Minimum Inhibitory Concentration (MIC) Selection

Susceptibility to antibiotics was also determined by broth macro-dilution. The MIC method by (Lin et al., 2014) was applied with slight modifications. A suitable range of antibiotic concentrations was chosen for the organisms to be tested. Suggested range for Enterobacteriaceae (*K. pneumoniae*) MIC determination (mg / L) is between 0.25 and 128 mg / L. Antibiotic ranges were prepared one step higher than the final dilution range required, to compensate for the addition of an equal volume of inoculum. Hence, antibiotic dilution range between 0.5 and 256 mg / L was prepared.

For the preparation of further stock solutions, from the initial 10 000 mg / L stock solution, the following was prepared.

1 mL of 10 000 mg/L solution + 9 mL diluent* = 1000 mg/L
100 µL of 10 000 mg/L solution + 9.9 mL diluent* = 100 mg/L
Diluent*: dH₂O

Ten sterile capped tubes were prepared in two rows for antibiotic Gentamicin to cover the range of antibiotic dilutions chosen in duplicate as shown below.

From the 10,000 mg / L stock, the following amount was dispensed with a micropipette:

Volume (µL)	Label (mg / L)
512	256
256	128
128	64
64	32
32	16

From the 1000 mg / L stock, the following amount was dispensed:

Volume (µL)	Label (mg / L)
160	8
80	4
40	2

From the 100 mg / L stock, the following amount was dispensed:

Volume (µL)	Label (mg / L)
200	1
100	0.5

One mL volumes of each antibiotic dilution were transferred in broth to the tubes. The inoculum was prepared following the procedures according to Section 2.5. A final inoculum of 10^5 cfu / mL is required and therefore suspensions should be diluted 1:100 in broth medium used for preparing the antibiotic dilutions. 1 mL aliquots of *K. pneumoniae* was added to one set of tubes and 1 mL of control organism to the other. The contents of the tubes were mixed thoroughly. Inoculated and uninoculated tubes of antibiotic-free broth were included as a positive control and check of sterility. The inoculum was incubated at 37 °C for 18-20 h. The MIC endpoint read as the lowest concentration of antibiotic at which there is no visible growth of bacteria.

2.11.3 Phage-Antibiotic Synergy (PAS)

The effectiveness of combining phages and antibiotics was determined by the time-kill curve method by Verma (2007) with slight modification. Changes in the bacterial count were monitored in a 15 mL centrifuge tube of three different conditions; a) single resistant bacteria (as to Section 2.5) that acts as a control, b) the combination of phages and sub-inhibitory concentration of antibiotics (as to Section 2.11.2) and c) phage cocktail infecting resistant *Klebsiella* strains (to Section 2.11.1). Respective culture conditions (a, b and c)

were added up to 10 mL as the final volume in a 15 mL centrifuge tube. The test tubes were incubated at 37 °C and bacteria growth OD₆₀₀ were determined by spectrophotometer after 0 h, 1 h, 2 h, 3 h and >18 h of incubation. The results from the experiments, carried out at least twice to obtain average readings. If bacterial growth reading decreased marginally for the phage-antibiotic combination compared to the more active single agent (phages), as well as to the initial inoculum titer, the interaction considered to be synergistic.

CHAPTER 3

RESULTS

3.1 Verification of *Klebsiella pneumoniae* Genomic DNA

3.1.1 DNA Fragments Visualisation by Agarose Gel Electrophoresis

Figure 3.1 and Figure 3.2 represent the result after agarose gel electrophoresis of PCR products *Klebsiella* spp. and ATCC KP was used as a positive control. Universal primers used were mentioned in Section 2.6.2. After separation, the resulting DNA fragments are visible as clearly defined bands. The DNA standard marker separated to a degree that allows for the useful determination of the sizes of sample bands. The *Klebsiella* spp. DNA fragments of approximately 1.5 kb are separated on a 1 % agarose gel along with a 1 kb DNA ladder.



Figure 3.1: Agarose gel electrophoresis of PCR product for universal primers 27F and 152R. Lane M indicates the Thermo Scientific 1kb DNA ladder. Lane 1 is a positive control using ATCC KP. Lane 12 is the negative (water) control. Lane 2: KP5, Lane 3: ESKP2, Lane 4: CRKP1, Lane 5: KP6, Lane 6: KP7, Lane 7: KP8, Lane 8: E. coli, Lane 9: KP9, Lane 10: ESKP3 and Lane 11: KP10.

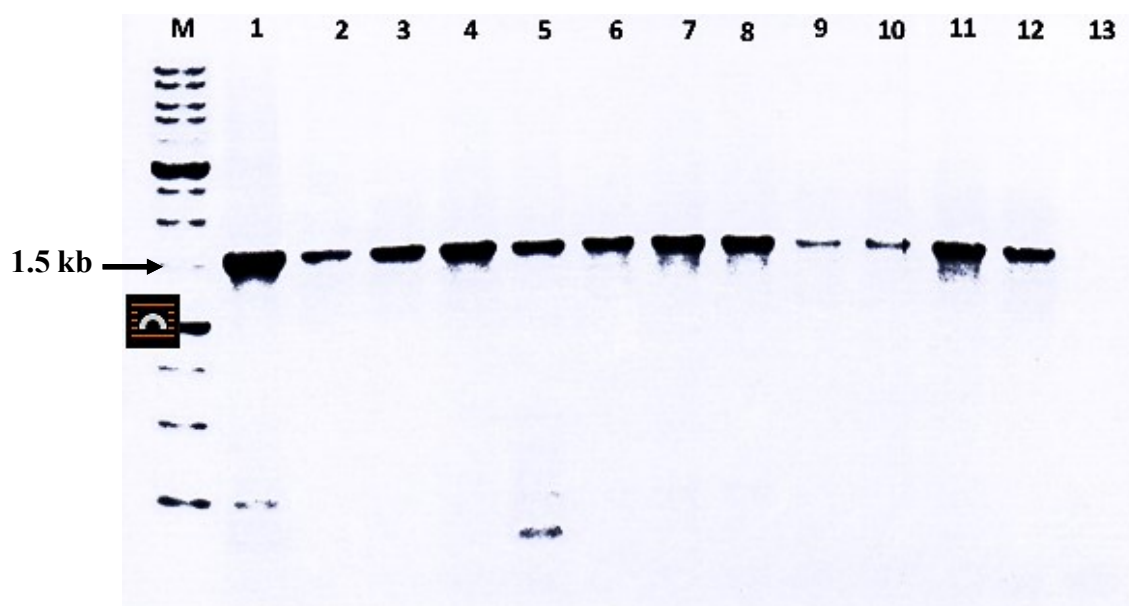


Figure 3.2: Agarose gel electrophoresis of PCR product for universal primers 27F and 152R. Lane M indicates the Thermo Scientific 1kb DNA ladder. Lane 1 is a positive control using *K. pneumoniae* ATCC 17978. Lane 13 is the negative (water) control. Lane 2: ESKP3, Lane 3: KP11, Lane 4: KP12, Lane 5: KP13, Lane 6: KA1, Lane 7: KP14, Lane 8: KA2, Lane 9: KP15, Lane 10: KP16, Lane 11: KP17, Lane 12: KO1

3.1.2 NCBI BLAST® Analysis

The samples were sent for Sanger sequencing according to Section 2.6.5 in which the isolates were validated for *K. pneumoniae*. However, only 78 % of the isolates with similarity value between 95-100 % were verified to be *K. pneumoniae* and 22 % were of other species such as *K. aerogenes*, *K. oxytoca*, and *E. coli*. In this study, the *E. coli* strain (Lane 8) in Figure 3.1 was omitted to focus only on those belonging to *Klebsiella* species. Therefore, total of twenty-one *Klebsiella* spp. strains were utilised including three extended-spectrum β -lactamases (ESBL) *K. pneumoniae* strains and one Carbapenem-resistant *K. pneumoniae* (CRKP) strain. Unavailable GenBank accession no. of certain strains were due to primer-dimer sequencing.

Table 3.1: The percentage of BLAST similarity of *Klebsiella* spp. 16S rRNA PCR sequences

No	Bacteria	Species	Percentage (%)	GenBank accession no.
1	CRKP1	<i>K. pneumoniae</i>	97	MK386770
2	ESKP2	<i>K. pneumoniae</i>	97	-
3	ESKP3	<i>K. pneumoniae</i>	98	MK386781
4	ESKP4	<i>K. pneumoniae</i>	99	-
5	KP5	<i>K. pneumoniae</i>	95	MK386771
6	KP6	<i>K. pneumoniae</i>	98	MK386786
7	KP7	<i>K. pneumoniae</i>	98	MK386785
8	KP8	<i>K. pneumoniae</i>	99	MK386783
9	KP9	<i>K. pneumoniae</i>	98	MK386782
10	KP10	<i>K. pneumoniae</i>	98	MK386784
11	KP11	<i>K. pneumoniae</i>	99	MK386779
12	KP12	<i>K. pneumoniae</i>	98	MK386778
13	KP13	<i>K. pneumoniae</i>	97	-
14	KP14	<i>K. pneumoniae</i>	99	MK386776
15	KP15	<i>K. pneumoniae</i>	96	MK386773
16	KP16	<i>K. pneumoniae</i>	98	MK386772
17	KP17	<i>K. pneumoniae</i>	96	MK386777
18	KA1	<i>K. aerogenes</i>	98	MK386775
19	KA2	<i>K. aerogenes</i>	98	MK386774
20	KO1	<i>K. oxytoca</i>	98	MK386769

3.2 Determination on *Klebsiella* spp. Log-phase

Figure 3.3 shows the average bacterial growth measurement (OD₆₀₀) for ATCC KP at the different growth phases after incubation in Luria-Bertani (LB) broth at 37 °C, shaking for 24 h with the procedure described in Section 2.5. Based on Figure 3.3 shown below, the log-phase (exponential growth) of ATCC KP starts to occur only after 3 h of incubation. Log-phase growth of ATCC KP act as a positive control, used to estimate the log-phase growth for the rest of *Klebsiella* spp. performed in this experiment.

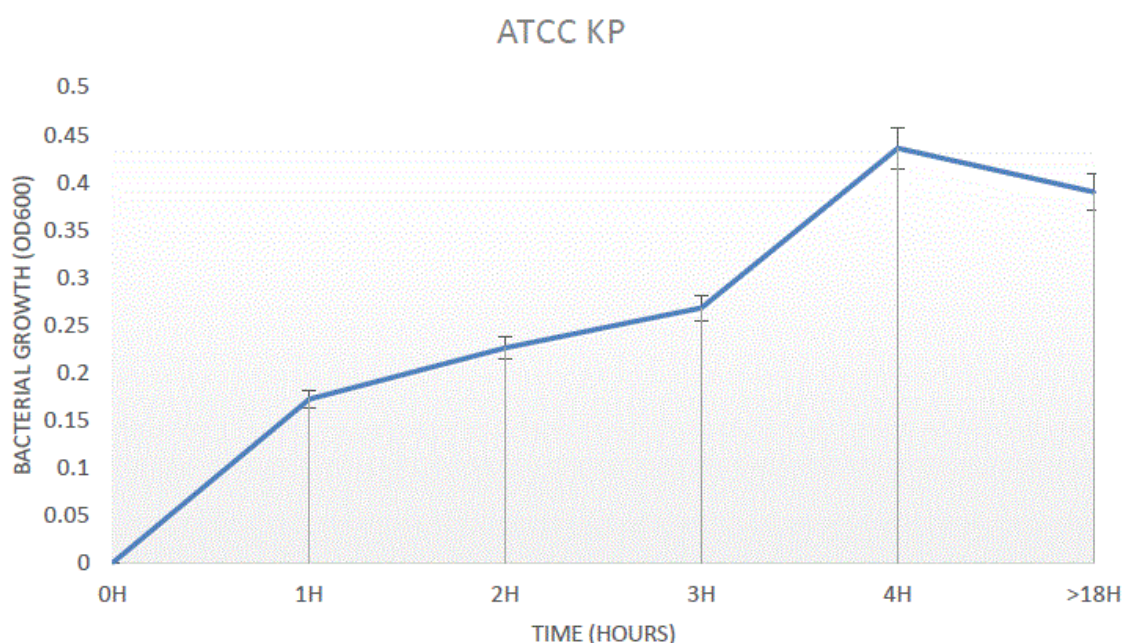


Figure 3.3: Growth curve of ATCC KP within 24 h using OD₆₀₀ measurements. Each point corresponds to the average of three determinations $\pm$ standard deviation.

3.3 Antibiotic Sensitivity Testing (AST) on *Klebsiella pneumoniae* Clinical Isolates

Most clinical isolates of *Klebsiella pneumoniae* were found to be increasingly resistant to many antibiotics, making it difficult on the choice of an adequate antibiotic for the treatment of *K. pneumoniae* infections. As an example, KP15 in Figure 3.4 showed small zones and no inhibition towards certain antibiotics as a sign of bacterial resistance. Despite the resistance problem, these antibiotics are still in use. Antibiotic sensitivity test as in Section 2.7 was assessed by the Kirby-Bauer disk diffusion method on Mueller-Hinton agar. In this study, seventeen antibiotics were used to determine *K. pneumoniae* antibiotic sensitivity.

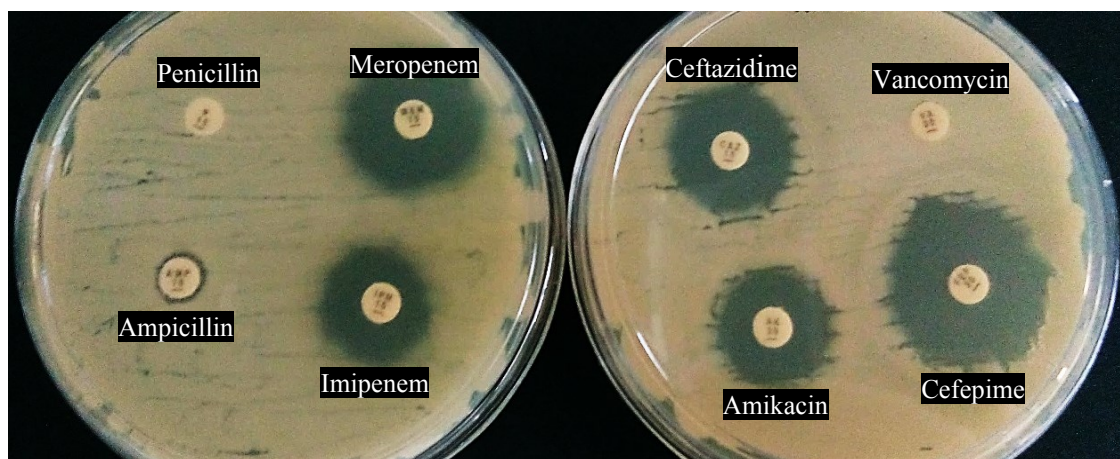


Figure 3.4: Antibiotic sensitivity test; Different antibiotic discs tested on *K. pneumoniae* KP15

Table 3.2 and Table 3.3 showed that *K. pneumoniae* strains have different resistant patterns towards different types of antibiotics. As mentioned previously in Section 2.3, AST on *K. pneumoniae*, clinical strains have been assessed by BMC, Kuching. Hence, only AST on *K. pneumoniae* community-acquired strains was conducted in UNIMAS lab. All the selected strains (100 %) showed resistance towards Ampicillin. Followed by 58.3 % resistance towards Trimethoprim / Sulfamethoxazole. *K. pneumoniae* CRKP1, being carbapenem-resistant showed 100 % resistance and intermediate effect towards 15 antibiotics tested. Carbapenem resistances are considered a major problem as carbapenems were often used as drugs of last resort for critical Gram-negative infections (Fair & Tor, 2014). Subsequently, *K. pneumoniae* ESBL-producing ESKP2, ESKP3 and ESKP4 also showed high resistance with 66.7 %, 72.7 % and 63.6 % towards all tested antibiotics, respectively. Nevertheless, five *K. pneumoniae* strains (KP6, KP7, KP8, KP11 & KP13) managed to achieve 90 % - 100 % susceptibility towards the fifteen antibiotics tested.

Table 3.2: Antibiotics sensitivity testing of *K. pneumoniae* clinical strains (CRKP1 to KP9)

Antibiotics	<i>Klebsiella pneumoniae</i> strains								
	CRKP1	ESKP2	ESKP3	ESKP4	KP5	KP6	KP7	KP8	KP9
Ampicillin									
Amikacin							S		
Amoxicillin	R	I	R	R	I	S	S	S	I
Sulbactam Ampicillin	R	R	R	R	R	S	S	S	R
Piperacillin-tazobactam	R	S		S	R			S	I
Cefuroxime (Oral)	R	R	R	R	S	S	S	I	S
Cefuroxime (IV)	R	R	R	R	S	S	S	S	S
Ceftazidime	R	R	R	R	S	S	S		
Ceftriaxone				R	S	S		S	
Cefepime	R	R	R	R	S	S	S	S	S
Imipenem	R	S	S	S	S	S	S	S	S
Meropenem	R	S	S	S	S	S	S	S	S
Gentamicin	I	R	R	S	R	S	S	S	R
Ciprofloxacin	I	R	S		R	S		S	R
Trimethoprim / Sulfamethoxazole	R	S	R		R	S	R	S	R

S=Sensitive, I=Intermediate, R=Resistance

Table 3.3: Antibiotics sensitivity testing of *K. pneumoniae* clinical and non-clinical strains (KP10 to ATCC KP)

Antibiotics	<i>Klebsiella pneumoniae</i> strains								
	Clinical					Non-clinical			Reference
	KP10	KP11	KP12	KP13	KP14	KP15	KP16	KP17	ATCC KP
Ampicillin	R		R		R	R	R	R	R
Amikacin	S	S	S	S		I	I	I	S
Amoxicillin	I	I		S	R				
Sulbactam Ampicillin	I	S		S	R				
Piperacillin-tazobactam				S	S				
Cefuroxime (Oral)	I	I		S	R				
Cefuroxime (IV)	S	S		S	R				
Ceftazidime	S	S	R	S	R	I	R	S	S
Ceftriaxone				S	I				
Cefepime	S	S	S	S	S	S	S	I	S
Imipenem			I		S	R	I	I	R
Meropenem			I		S	I	I	S	R
Gentamicin	S	S	S	S	S	S	S	S	S
Ciprofloxacin	S	S		S	R				
Trimethoprim / Sulfamethoxazole	R	S		S	R				

S=Sensitive, I=Intermediate, R=Resistance

3.4 Housekeeping Genes Sequencing Analysis of *Klebsiella pneumoniae*

The bacterial species was confirmed by phylogenetic analysis of three-loci concatenated genes; *rpoB* (501 bp), *gapA* (450 bp), and *tonB* (414 bp). For phylogenetic analyses, a maximum-likelihood phylogenetic tree was generated by MEGA 6 with a Tamura-Nei model and 1000 bootstrap replications with the results as shown in Table 3.4. Phylogenetic trees of *rpoB* gene, *gapA* gene and *tonB* gene were constructed as shown in Figure 3.5 to Figure 3.7.

Table 3.4: Sequence query of *Klebsiella* locus under MLST scheme

Strains	gapA	rpoB	tonB
CRKP1	1	1	12
ESKP2	3	4	4
ESKP3	4	4	46
ESKP4	2	10	12
KP5	2	1	6
KP6	2	4	1
KP7	2	4	42
KP8	2	4	4
KP9	2	1	26
KP10	2	4	12
KP11	9	1	27
KP12	2	4	254
KP13	2	4	12
KP14	2	1	16
KP15	2	1	129
KP16	94	13	51
KP17	2	8	24
KA1	168	164	304
ATCC KP	2	4	12

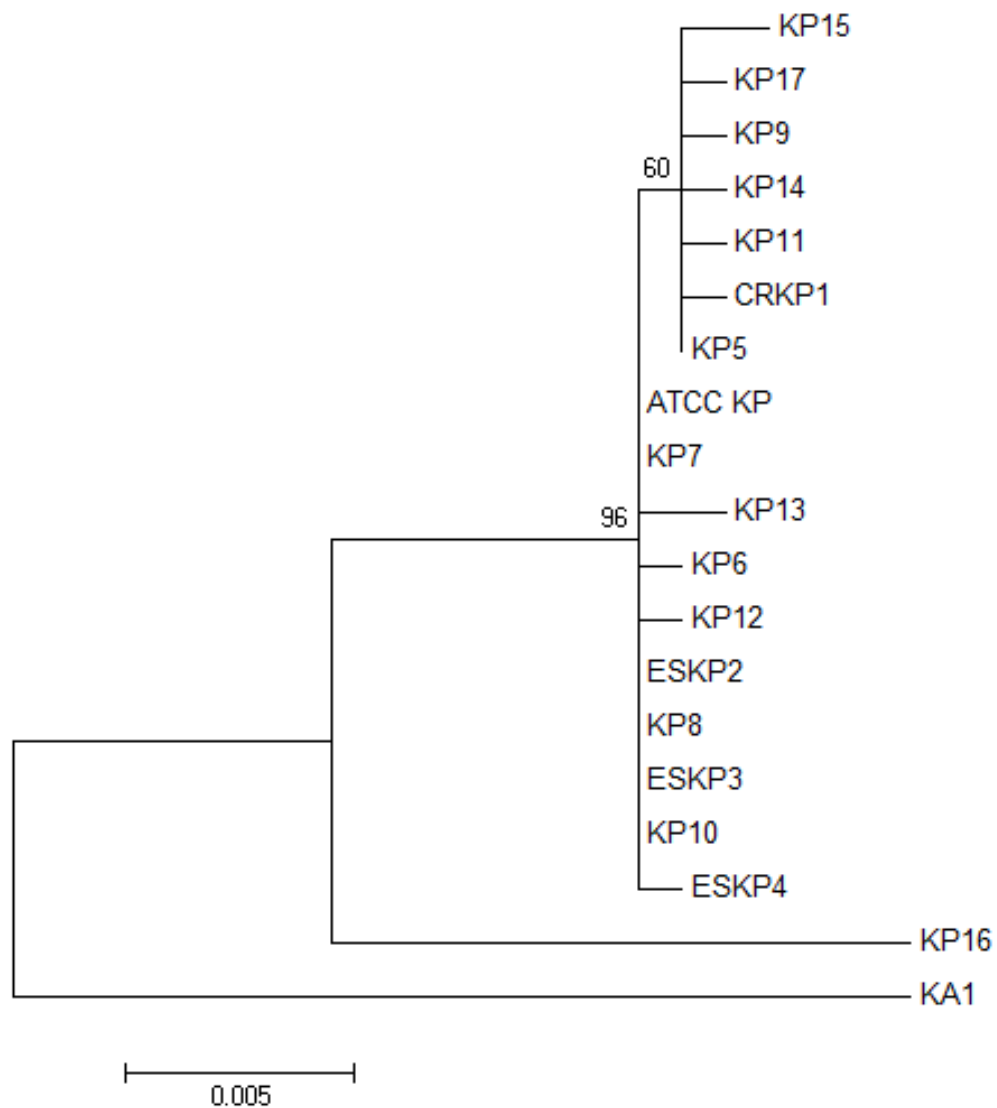


Figure 3.5: Phylogenetic tree resulting from Maximum Likelihood Method for *Klebsiella pneumoniae* rpoB gene using Mega 6 software. Display of node statistics from 1,000 bootstrap replications. KA1 belonging to *K. aerogenes* is used as a reference strain. Bar, 0.5% nucleotide change.

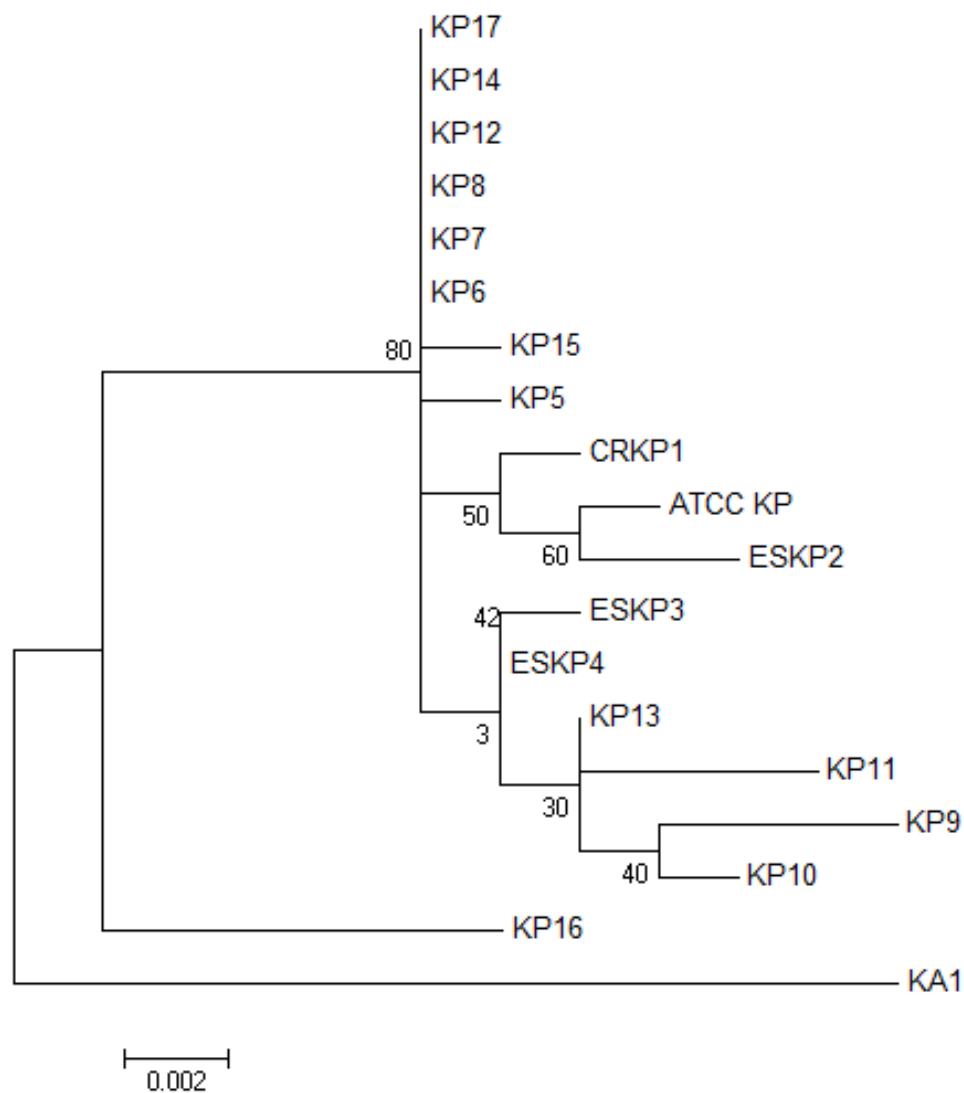


Figure 3.6: Phylogenetic tree resulting from Maximum Likelihood Method for *Klebsiella pneumoniae* gapA gene using Mega 6 software. Display of node statistics from 1,000 bootstrap replications. KA1 belonging to *K. aerogenes* is used as a reference strain. Bar, 0.2% nucleotide change.

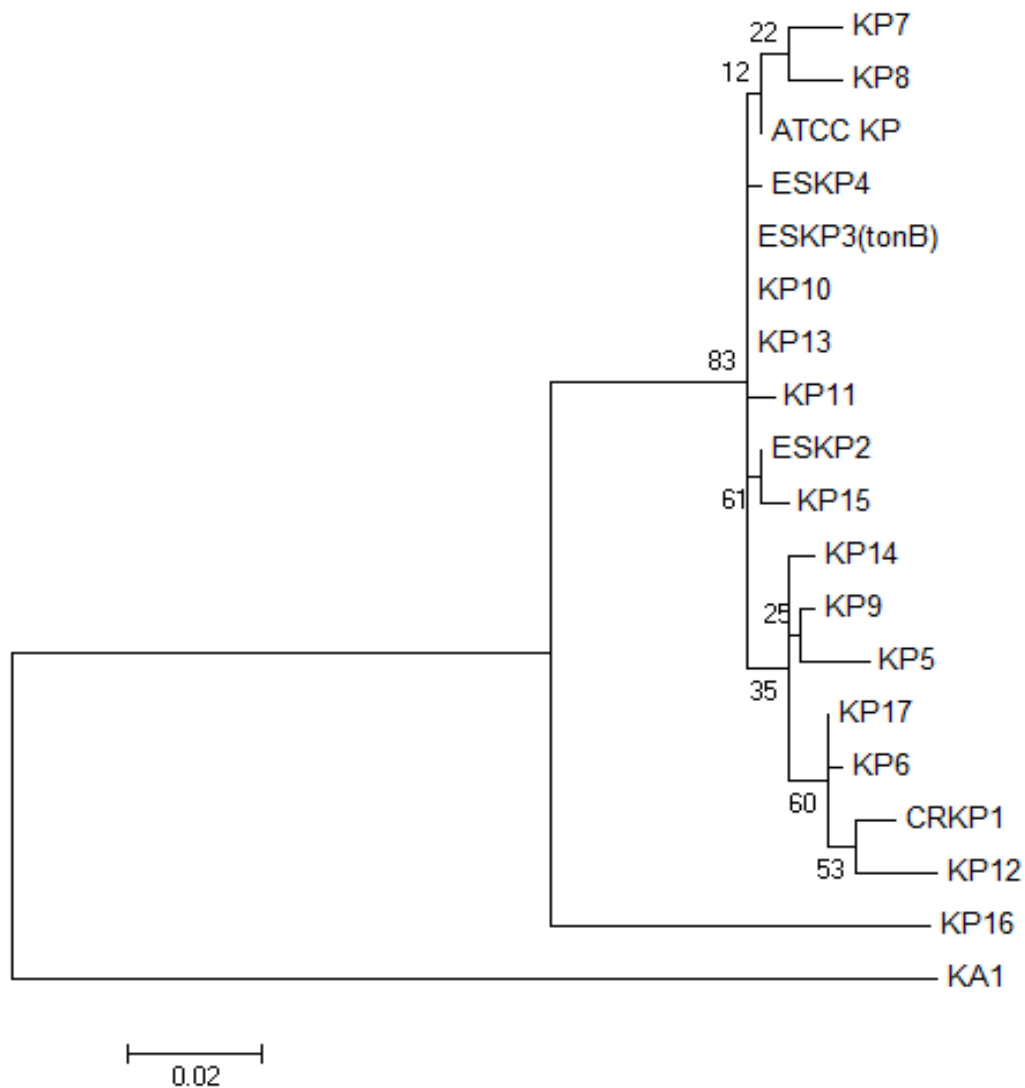


Figure 3.7: Phylogenetic tree resulting from Maximum Likelihood Method for *Klebsiella pneumoniae* tonB gene using Mega 6 software. Display of node statistics from 1,000 bootstrap replications. KA1 belonging to *K. aerogenes* is used as a reference strain. Bar, 2% nucleotide change.

Based on above analysis, KP16 has the highest possibility belonging to ST1484 since the combination of loci (gapA: 94, rpoB: 13 and tonB: 51) managed to generate only one result from the *Klebsiella* MLST database. Whereas the combination of loci for the rest of *Klebsiella* strains generates multiple results. Hence, it is acceptable to deduce that KP16 belongs to ST1484.

3.5 Bacteriophages Isolation, Purification and Titration

Bacteriophages were isolated as described in Section 2.9.3 and Section 2.9.4 by serial dilution and plaque assay, respectively. In total, eleven *Klebsiella* phages, all of which were isolated on and have lytic activity against *Klebsiella* strains as shown in Table 3.5. All were virulent phages isolated from the environmental sample, originating from sewage influent. These samples were first suspended in LB broth and then filtered to remove bacteria. This filtrate was then incubated with three individual *K. pneumoniae* strains to amplify any phage present (as to Section 2.9.2). The three *K. pneumoniae* strains used were CRKP1, KP17, and ATCC KP. All phages were purified through three rounds of plaque purification. Following plaque purification, high titer phage stocks were produced and enumerated by limiting dilution.

3.5.1 Bacteriophages Serial Dilution

Enriched bacteriophages (as to Section 2.9.2) were diluted by serial dilution method (as to Section 2.9.3) to isolate different types of phages in the sample for plaque purification to obtain homogenous phage plaques. Figure 3.8 shows three types of phages with different morphologies were isolated from bacterial host (KP17) in dilution plate 10^{-5} . Only clear plaques (Plaque A) were chosen for the next round of purification as it shows greater lytic property and highly reproducible.

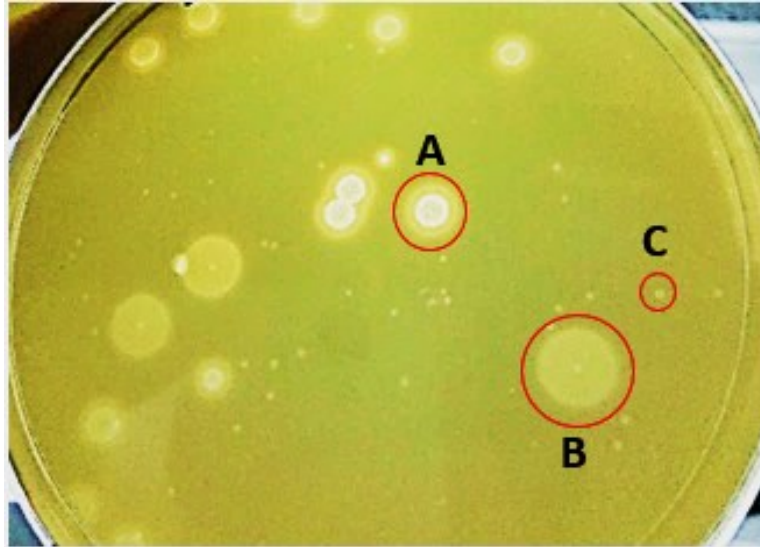


Figure 3.8: Three different phage plaque morphologies (A, B and C) infecting host KP17.

3.5.2 Step-by-Step (SBS) Method

A phage cocktail lytic for *Klebsiella pneumoniae* in Section 2.11.1 was established by the SBS method. This method takes advantage of the occurrence of phage-resistant bacteria variants and ensures that phages lytic for wild-type strain and its phage-resistant variants are selected. Therefore, different bacteria were used as host strains to attain different bacteriophages that were able to lyse the phage-resistant bacteria variants shown in Table 3.5. Eleven different bacteriophages were able to be isolated from the SBS method and further characterised in the next sections.

Table 3.5: Bacteriophages isolated using different host strains respectively by SBS method

Phage	Host Bacterial Strain (<i>K. pneumoniae</i>)
ϕ KPaV03	ATCC KP
ϕ KPaV04	CRKP1
ϕ KPaV05	KP7
ϕ KPaV06	KP14
ϕ KPaV07	KP6
ϕ KPaV08	KP17
ϕ KPaV09	KP17
ϕ KPaV10	ATCC KP
ϕ KPaV12	KP15
ϕ KPaV13	KP16
ϕ KPaV14	ESKP4

3.5.3 Bacteriophage Host Range Analysis

One of the goals of this study was to determine the specificity of phage on bacterial isolates, with the expectation that some phage would have a broader tropism than others due to presence or absence of phage receptor molecules. To assess the tropism of the phages shown in Table 3.5, spot assays described in Section 2.9.5 were performed on plates containing lawns of various *K. pneumoniae* isolates. Plates were assessed for the lytic ability of each phage isolate by monitoring for clearing of bacterial lawns shown in Figure 3.9 and all spot tests were repeated twice to confirm the validity of the results. All bacterial isolates in Table 2.2 were assessed for phages host range, although some isolates were not lysed by any of the phages, thus those results were omitted from Table 3.5.

Based upon spot assay results, at least 5 unique phages were isolated: ϕ KPaV03, ϕ KPaV04, ϕ KPaV08, ϕ KPaV10, ϕ KPaV12. Phage ϕ KPaV03 has the broadest host range, with the lytic ability against nine *Klebsiella* strains including one *Klebsiella aerogenes* that makes up 50 % of bacterial lysis. ϕ KPaV06, ϕ KPaV07 and ϕ KPaV09 have the narrowest

host range, with only 2 different bacterial strains being lysed. Besides phage ϕ KPaV03, other phages that were able to lyse more than three bacterial strains namely ϕ KPaV04, ϕ KPaV08, ϕ KPaV10 and ϕ KPaV12 were chosen to produce phage cocktail as described in Section 2.11.1.

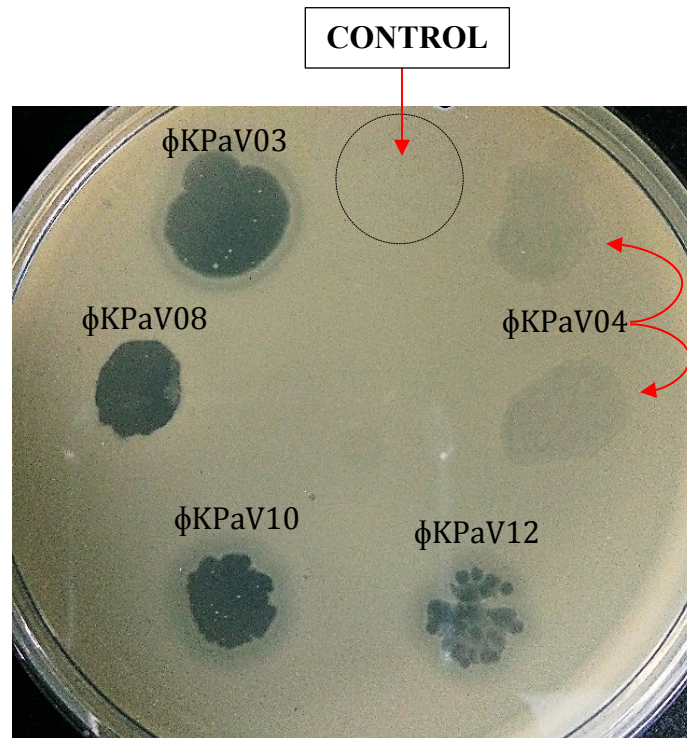


Figure 3.9: Spot assay of different phages in clockwise direction: ϕ KPaV04, ϕ KPaV12, ϕ KPaV10, ϕ KPaV08, ϕ KPaV03 and water (control) spotted on bacterial host KP10.

Table 3.6: Assessment of phages host range by spot assay

Bacterial Strain	ϕ KPaV03	ϕ KPaV04	ϕ KPaV05	ϕ KPaV06	ϕ KPaV07	ϕ KPaV08	ϕ KPaV09	ϕ KPaV10	ϕ KPaV12	ϕ KPaV13	ϕ KPaV14
1. ATCC KP											
2. CRKP1											
3. ESKP2											
4. ESKP3											
5. ESKP4											
6. KP5											
7. KP6											
8. KP7											
9. KP8											
10. KP9											
11. KP10											
12. KP11											
13. KP12											
14. KP13											
15. KP14											
16. KP15											
17. KP16											
18. KP17											

Table 3.6 above shows the host ranges of *Klebsiella* phages. Phage isolates are indicated at the top, and host bacterial strains are indicated on the left. The extent of lysis is indicated by colours, as follows: Black for clear plaques and White for no plaques or turbid plaques.

3.5.4 Bacteriophage Titer

Bacteriophage titration was used to quantify the number of phage in a sample expressed as plaque-forming unit (pfu) / mL. Serial dilution was done for the phage lysate from 10^{-1} to 10^{-9} as described in Section 2.9.3 and the morphology of the plates was then examined to make sure the plaques were all homogenous. The plate that had between 30 and 300 plaques was selected for counting based on Equation 2.1. For dilution of 10^{-1} and 10^{-2} , the agar plates were all clear and no plaques were shown in all isolated phages. ϕ KPaV12 has the highest titer of 1.4×10^{12} pfu / mL and ϕ KPaV04 with the lowest titer of 4.0×10^8 pfu / mL.

Table 3.7: Average titer (pfu / mL) of phage isolates

Phage Isolate	Highest Dilution	Average pfu / mL
ϕ KPaV03	10^{-6}	3.6×10^9
ϕ KPaV04	10^{-5}	4.0×10^8
ϕ KPaV08	10^{-7}	4.2×10^{10}
ϕ KPaV10	10^{-6}	1.0×10^9
ϕ KPaV12	10^{-8}	1.4×10^{12}

3.6 Bacteriophage Characterisation

3.6.1 Plaque Morphology

Based on Figure 3.11, phages ϕ KPaV03 and ϕ KPaV04 produced approximately 1-2 mm clear plaques, while phages ϕ KPaV08 and ϕ KPaV12 produced clear plaques approximately 3–5 mm in diameter surrounded by a small halo with the exception being phage ϕ KPaV10, which produced large plaques (6–7 mm) bull's eye morphology. Plaque morphology of all phages was determined using the same medium and conditions; thus the differences resulted from the properties of each phage.

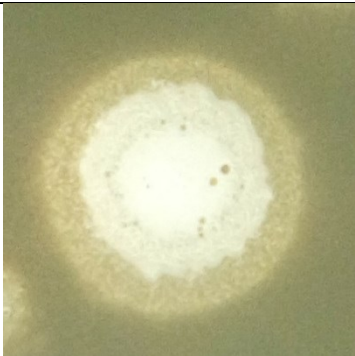
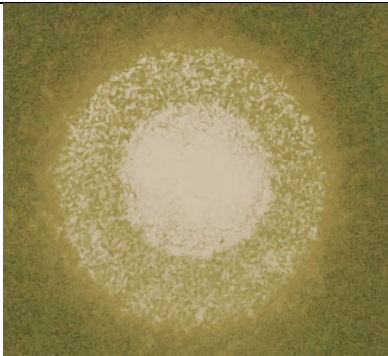
Phage ϕ KPaV03	Phage ϕ KPaV04
	
Morphology: Turbid ring with clear center; Diameter: 1.5-2.0 mm	Morphology: Clear; Diameter: 0.5–1.0 mm
Phage ϕ KPaV08	Phage ϕ KPaV10
	
Morphology: Turbid ring with clear center; Diameter: 2–3 mm	Morphology: Bull's eye; Diameter: 2–6 mm
Phage ϕ KPaV12	
	
Morphology: Clear; Diameter: 2-3 mm	

Figure 3.10: Different plaque morphologies formed by the isolated phages infecting *Klebsiella pneumoniae* strains from sewage influent

3.6.2 Multiplicity of Infection (MOI)

Table 3.8 shows the percentages of plaque formation on agar plates to increasing doses of phage dilution or in increasing MOI. The optimum MOI of ϕ KPaV03, ϕ KPaV04, ϕ KPaV08, ϕ KPaV10 and ϕ KPaV12 was determined by identifying the best ratio of bacteria to phages which was carried out according to Section 2.10.4. In this case, the optimal MOI for ϕ KPaV08 and ϕ KPaV12 is 0.01 whereas MOI for ϕ KPaV10 is 0.1 and for ϕ KPaV03 and ϕ KPaV04 are 1 and 10, respectively.

Table 3.8: Plaque assay analysis of *K. pneumoniae* clinical strains infected with increasing doses of isolated *Klebsiella* phages dilution (MOI). Values represent the average percentage of duplicate.

MOI	ϕ KPaV03	ϕ KPaV04	ϕ KPaV08	ϕ KPaV10	ϕ KPaV12
0.01	25	0	100	85	100
0.10	40	10	80	100	100
1.00	100	45	60	100	100
10.0	100	100	40	100	100

3.6.3 Single-Step Growth Curve

The life cycle and adsorption affinity of phage ϕ KPaV03, ϕ KPaV04, ϕ KPaV08, ϕ KPaV10 and ϕ KPaV12 were assessed when growing in *K. pneumoniae* host strain ATCC KP, CRKP1, KP17, and KP15, as shown in Figure 3.11 to Figure 3.15, respectively in LB broth medium at 37 °C. The single-step growth studies were performed to identify the different phases of a phage infection process.

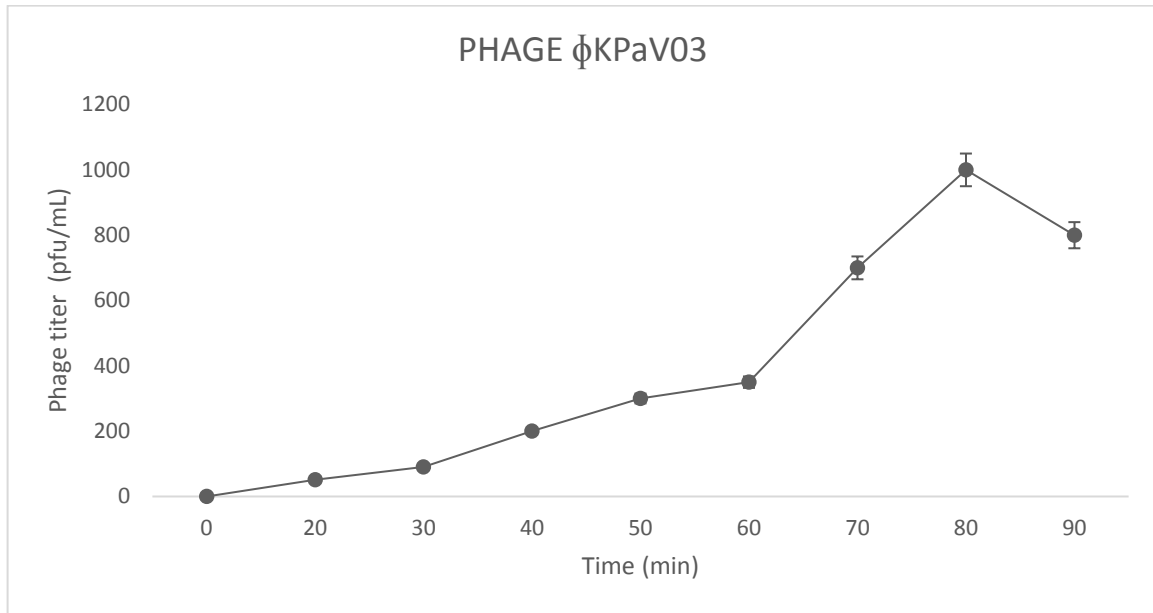


Figure 3.11: The single-step growth curve assay for bacteriophage ϕ KPaV03 infecting ATCC KP at MOI 1. The latent period takes less than 20 mins. Error bar indicates standard deviation.

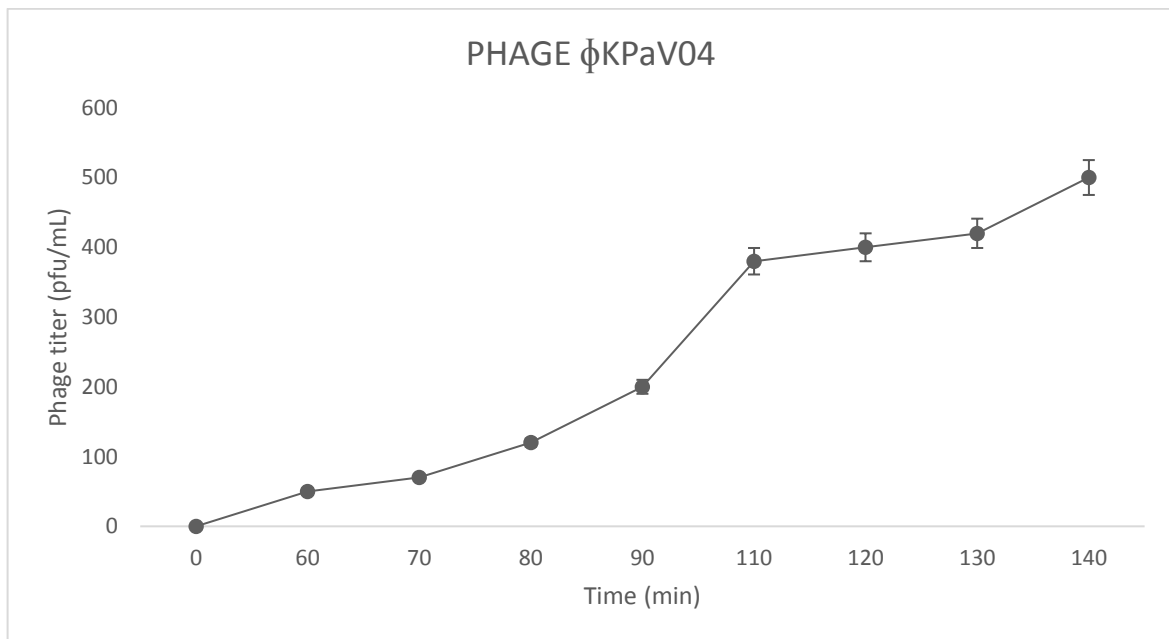


Figure 3.12: The single-step growth curve assay for bacteriophage ϕ KPaV04 infecting CRKP1 at MOI 10. The latent period takes approximately 60 mins. Error bar indicates standard deviation.

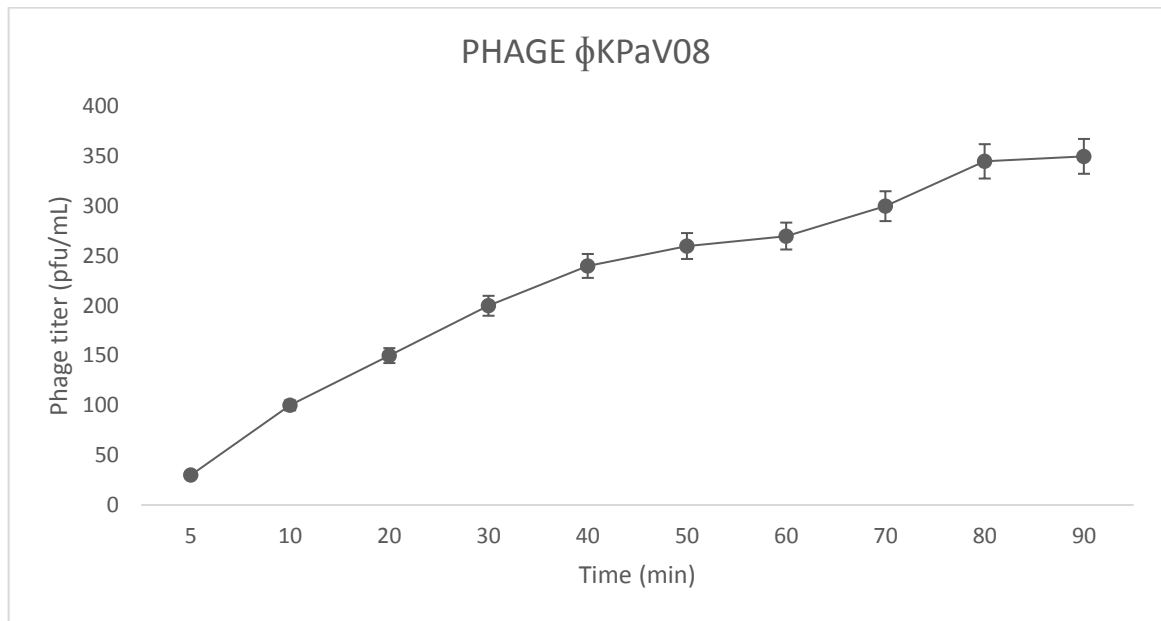


Figure 3.13: The one-step growth curve assay for bacteriophage ϕ KPaV08 infecting KP17 at MOI 0.01. The latent period takes less than 5 mins. Error bar indicates standard deviation.

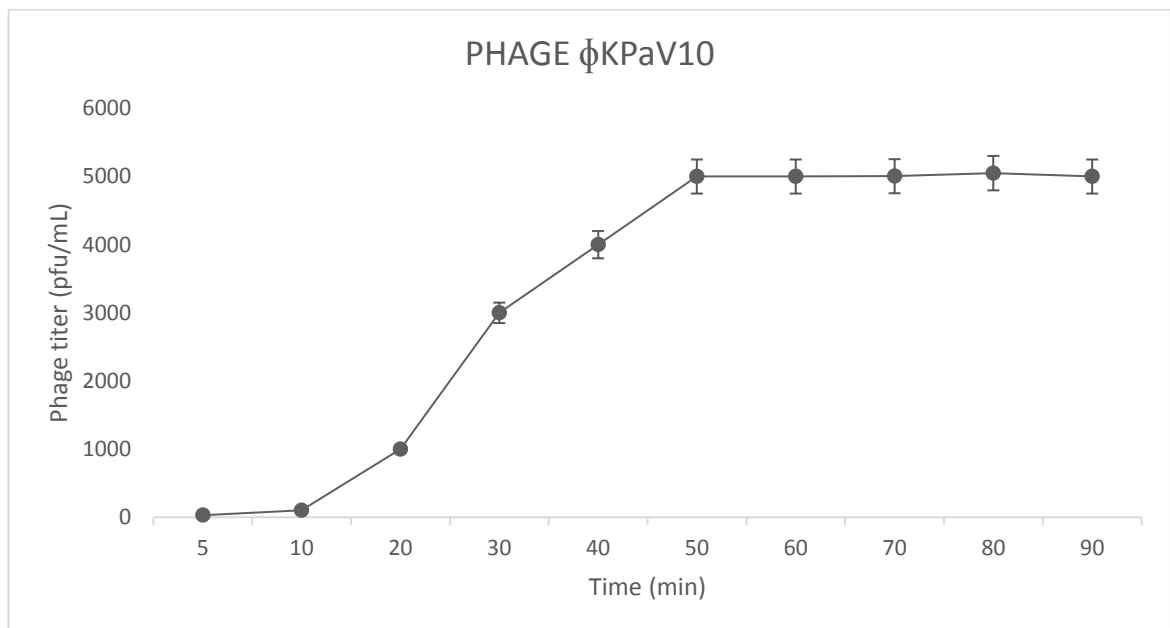


Figure 3.14: The one-step growth curve assay for bacteriophage ϕ KPaV10 infecting ATCC KP at MOI 0.1. The latent period takes approximately 20 mins. Error bar indicates standard deviation.

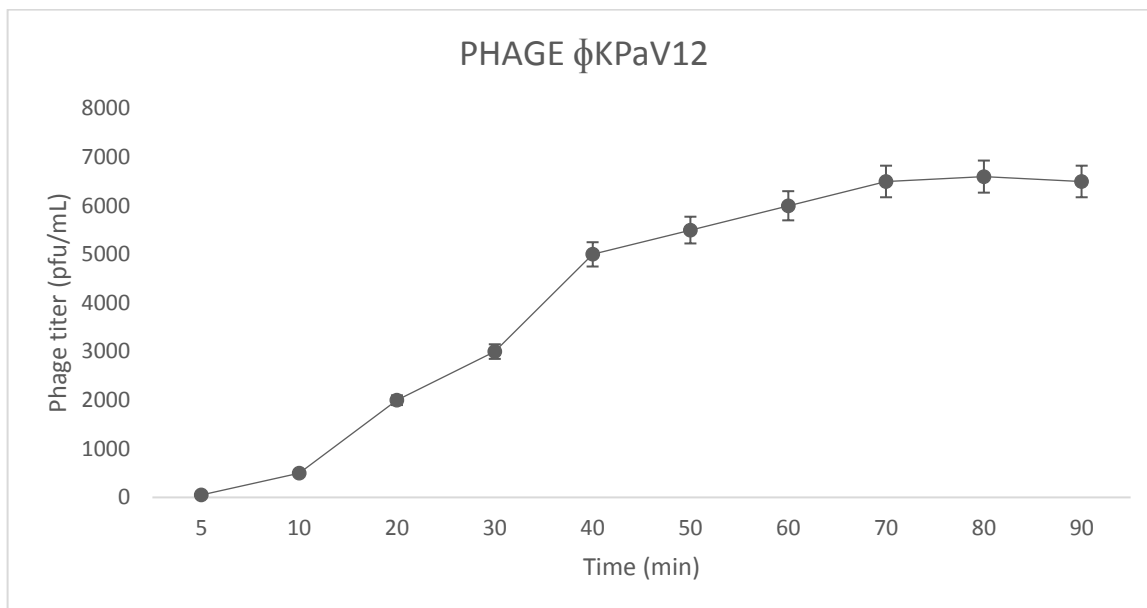


Figure 3.15: The one-step growth curve assay for bacteriophage ϕ KPaV12 infecting KP15 at MOI 0.01. The latent period takes less than 10 mins. The error bars indicated for standard deviation.

3.6.4 Bacteriophages Structural Protein Extraction

PCR amplification was conducted to determine the partial sequence of selected genes of the phages. The phage genome was successfully extracted and amplified based on the method described in Section 2.10.7. There were four pairs of primers used to target the different genes shown in Table 2.4. The genes targeted are lysin gene, viral capsid assembly protein gene (g20), major capsid protein gene (g23) and tail fiber protein gene.

The primer set targeting for lysin gene, major capsid protein gene (g23) and tail fiber protein gene, showed positive result on the electrophoresis gel photo as seen in Figure 3.16, Figure 3.17 and Figure 3.18, respectively. However, primer set targeting viral capsid assembly protein gene (g20) gene revealed no band on the electrophoresis gel for any of the 5 isolated bacteriophages. Hence, the gel photo result was omitted. The results were summarised in Table 3.9.

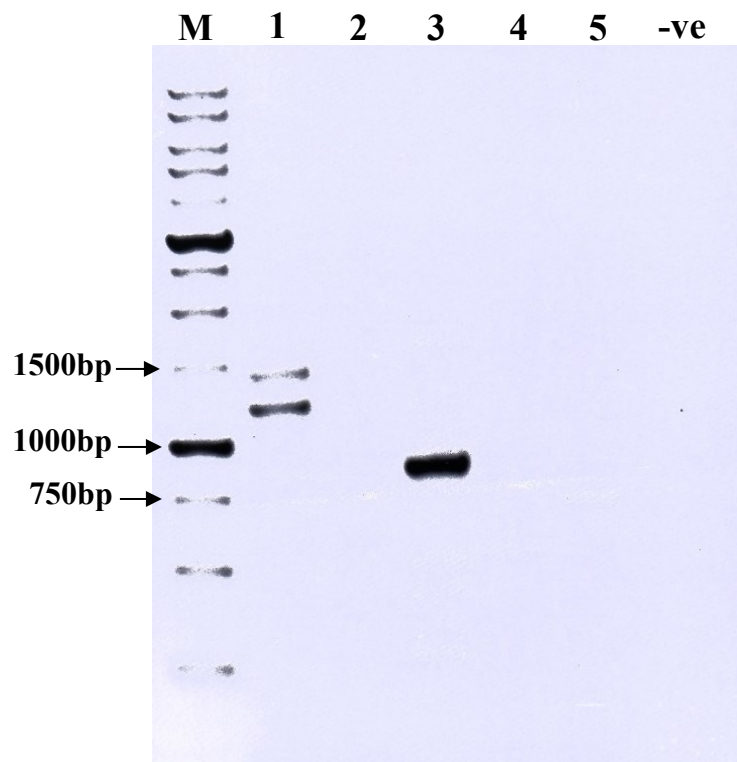


Figure 3.16: PCR products of Lysin gene (Lys) – Lane 1: ϕ KPaV03, Lane 2: ϕ KPaV04, Lane 3: ϕ KPaV08, Lane 4: ϕ KPaV10, Lane 5: ϕ KPaV12. Bacteriophage ϕ KPaV03 showed a clear band between 1.5kb and 1kb; bacteriophage ϕ KPaV08 showed a clear band between 1000bp and 750bp. Reference 1kb Promega DNA ladder in lane M was used.

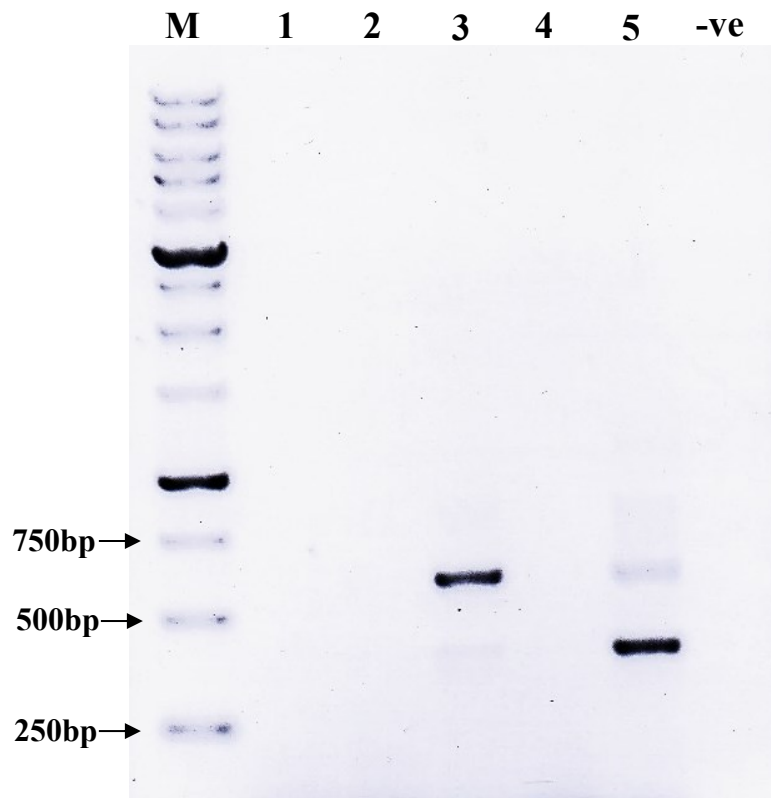


Figure 3.17: PCR products of major capsid protein gene (g23) – Lane 1: ϕ KPaV03, Lane 2: ϕ KPaV04, Lane 3: ϕ KPaV08, Lane 4: ϕ KPaV10, Lane 5: ϕ KPaV12. Bacteriophage ϕ KPaV08 showed a clear band between 750bp and 500bp; bacteriophage ϕ KPaV12 showed a clear band between 500bp and 250bp. Reference 1kb Promega DNA ladder in lane M was used.

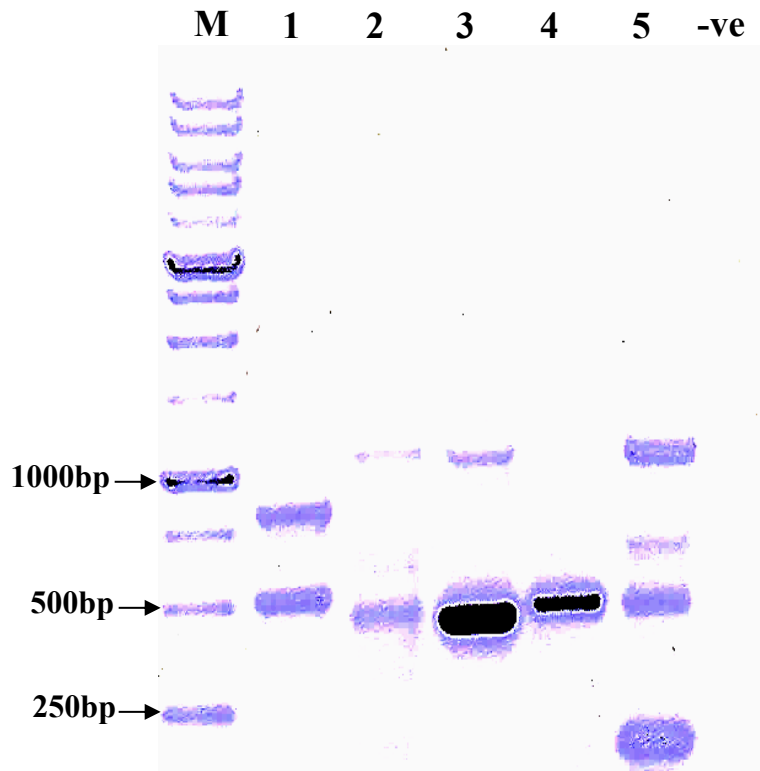


Figure 3.18: PCR products of tail fiber gene (T1)– Lane 1: ϕ KPaV03, Lane 2: ϕ KPaV04, Lane 3: ϕ KPaV08, Lane 4: ϕ KPaV10, Lane 5: ϕ KPaV12. Bacteriophages ϕ KPaV08 and ϕ KPaV10 showed a clear band between 500bp and 250bp; however, bacteriophages ϕ KPaV03, ϕ KPaV04 and ϕ KPaV12 showed multiple faint bands between 1000bp and 250bp. Reference 1kb Promega DNA ladder in lane M was used.

Table 3.9: Summary of amplification of each isolated phage structural protein genes

Phage	Structural protein genes		
	Lysin Gene	Major Capsid Protein (g23)	Tail Fiber Gene (T1)
ϕ KPaV03	✓	X	✓
ϕ KPaV04	X	X	✓
ϕ KPaV08	✓	✓	✓
ϕ KPaV10	X	X	✓
ϕ KPaV12	X	✓	✓

✓ - successful amplification of phage structural protein using specific primers

3.6.5 Bacteriophages Structural Protein Sequencing Analysis by UniProt Blast

The result of bacteriophage structural protein extracted as described in Section 2.10.7 was submitted for sequencing analysis. In which the sequencing results revealed that all the isolated phages belong to *Klebsiella* phages except ϕ KPaV10 which had similarity to *E. coli* phage. The information obtained from The Universal Protein Resource (UniProt) <https://www.uniprot.org/blast/> has been tabulated in Table 3.10.

Table 3.10: Characteristics of sequenced *Klebsiella* phages

No	Phage	Family	% identities	Sequence similarity	Reference
1	ϕ KPaV03	dsDNA viruses, Podoviridae	91	Putative peptidase of <i>Klebsiella</i> phage VB_KpnP_KpV48	https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=1912319
2	ϕ KPaV04	dsDNA viruses, Myoviridae	48	Ribonucleotide reductase, beta-subunit <i>Klebsiella</i> phage KP179	https://www.uniprot.org/taxonomy/2315700
3	ϕ KPaV08	dsDNA viruses, Myoviridae	99	Major capsid protein <i>Klebsiella</i> phage JD18	https://www.genome.jp/virushostdb/1698360
4	ϕ KPaV10	dsDNA viruses, Podoviridae	37	Tail Protein <i>E. coli</i> phage	-
5	ϕ KPaV12	dsDNA viruses, Myoviridae	97	Major capsid protein <i>Klebsiella</i> phage KPV15	https://www.uniprot.org/taxonomy/1913572

3.7 Phage Cocktail and Phage-Antibiotic Synergy approaches against Antibiotic Resistant *Klebsiella pneumoniae*

3.7.1 Phage Cocktail

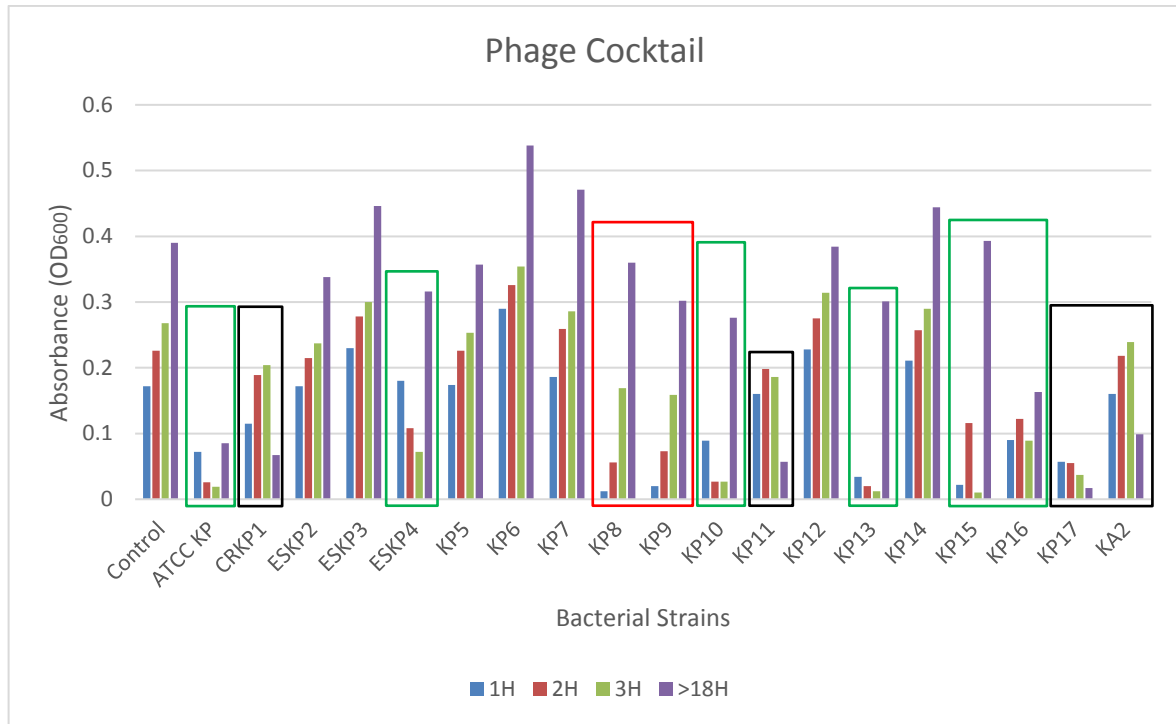


Figure 3.19: Phage cocktail approach against *Klebsiella* spp. strains. Results show three trends as depicted in the outlined boxes, **Black:** Reduction in bacterial growth after >18H of incubation; **Red:** Reduction in bacterial growth in the 1st hour of incubation; **Green:** Reduction in bacterial growth within the 3rd hour of incubation.

Based on Figure 3.20, three growth (OD₆₀₀) trends were observed. These trends were highlighted in black, green and red to describe the growth differences of *K. pneumoniae* strains when co-cultured with phage cocktail. Firstly, co-cultured bacterial strains outlined in black consist of CRKP1, KP11, KP17 including KA2 which belongs to *K. aerogenes* showed a significant decrease of bacterial growth $OD_{600} \leq 0.1$ after 18 h of incubation.

Secondly, trends outlined in green that belongs to *K. pneumoniae* strain ATCC KP, ESKP4, KP10, KP13, KP15 and KP16 showed a gradual decrease in $OD_{600} < 0.1$ during the first 3 h phage cocktail treatment. However, the bacterial growth began to increase after an overnight incubation.

Lastly, *K. pneumoniae* strains outlined in red consisting of strain KP8 and KP9 showed a drastic decrease ($OD_{600} < 0.05$) within the first hour of incubation from the initial OD_{600} of 0.1. Despite that, their growth managed to increase steadily throughout the incubation period.

As growth control, *K. pneumoniae* ATCC KP was cultured in the absence of phage showed a consistent increase in OD_{600} examined at different time points similar to Figure 3.3 in Section 3.4.

3.7.2 Minimum Inhibitory Concentration (MIC) selection

Klebsiella pneumoniae strains that showed resistance or intermediate effect towards Gentamicin (as to Table 3.2 and Table 3.3) were chosen for MIC selection. This is to determine the sub-inhibitory concentration of antibiotic to be applied in Section 2.11.3. To be considered as resistant, the MIC of *K. pneumoniae* strains must be higher than 2 $\mu\text{g} / \text{mL}$. This study as validated and proven that the selected strains CRKP1, ESKP2, ESKP3, KP5 and KP9 have complete or intermediate resistance towards Gentamicin (as to Table 3.11).

Table 3.11: Minimum inhibitory concentration (MIC) of Gentamicin-resistant *Klebsiella pneumoniae* strains

<i>K. pneumoniae</i> strain	MIC (µg / mL)	Sub-inhibitory concentration (µg / mL)	Susceptibility
CRKP1	256	128	R
ESKP2	>256	256	R
ESKP3	4	2	I
KP5	>256	256	R
KP9	>256	256	R

R=Resistant; I=Intermediate

3.7.3 Comparison of Phage Cocktail and Phage-Antibiotic Synergy approaches

To an initial inoculum of OD₆₀₀, the cocktail of five bacteriophages, as well as phage-antibiotic concentration, was added at MOI 1, to study its impact on the growth kinetic of Gentamicin resistant *K. pneumoniae* strains listed in Table 3.11 with time. *K. pneumoniae* ATCC KP act as a positive control.

According to the time-kill curves, as shown in Figure 3.20 to Figure 3.25, phage-cocktail (PC) approach managed to decrease the growth of all *K. pneumoniae* gentamicin-resistant strains except for ESKP3. As for Phage-Antibiotic (PA) synergy, it can be observed that 4 out of 5 gentamicin-resistant strains growth declined up until the 3rd hour of incubation. However, only CRKP1 showed a completed growth reduction after 18 h, having similar trend to the growth of *K. pneumoniae* ATCC KP that function as the positive control.

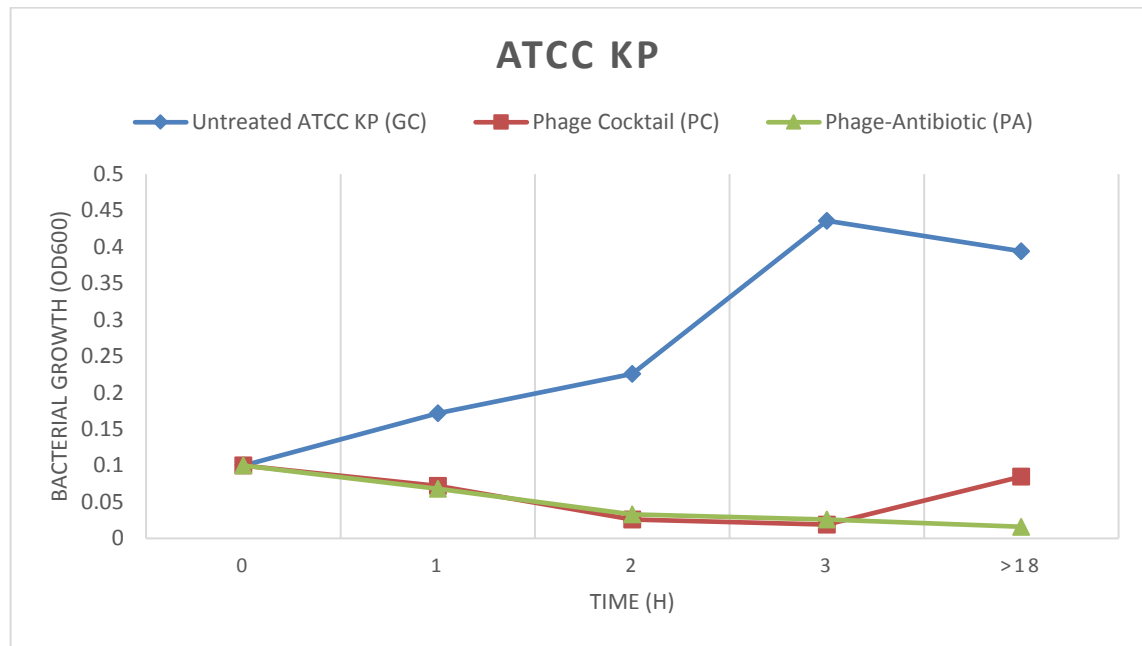


Figure 3.20: Time-kill curves of phage cocktail (PC), phage-antibiotic synergy when treated with sub-inhibitory concentration of Gentamicin (PA) and growth control curve (GC) versus ATCC KP.

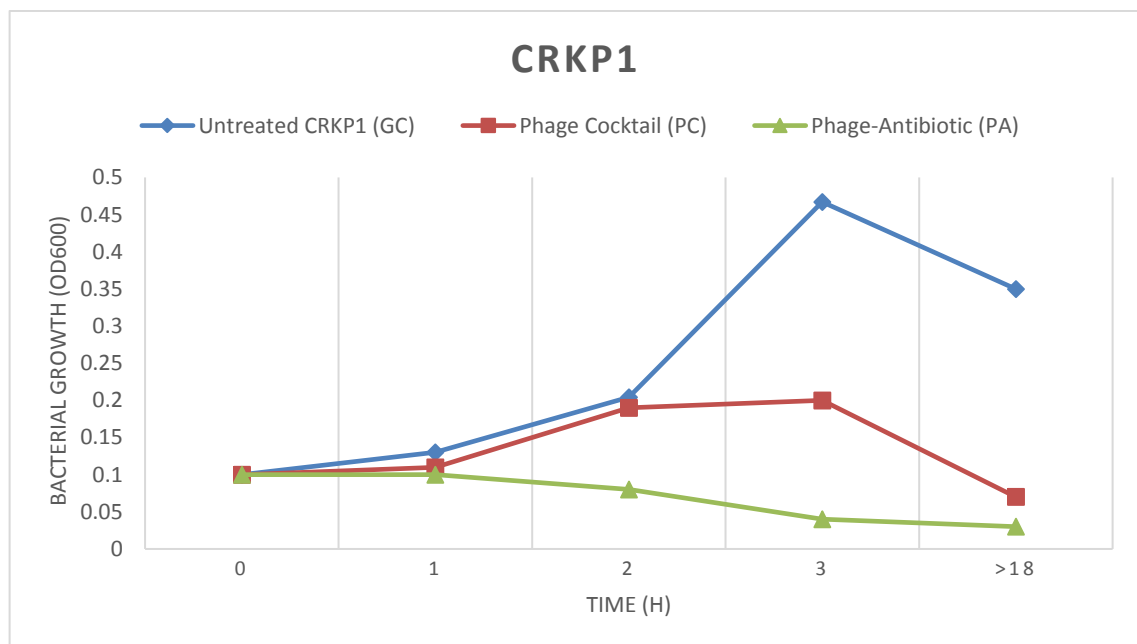


Figure 3.21: Time-kill curves of phage cocktail (PC), phage-antibiotic synergy when treated with sub-inhibitory concentration of Gentamicin (PA) and growth control curve (GC) versus CRKP1.

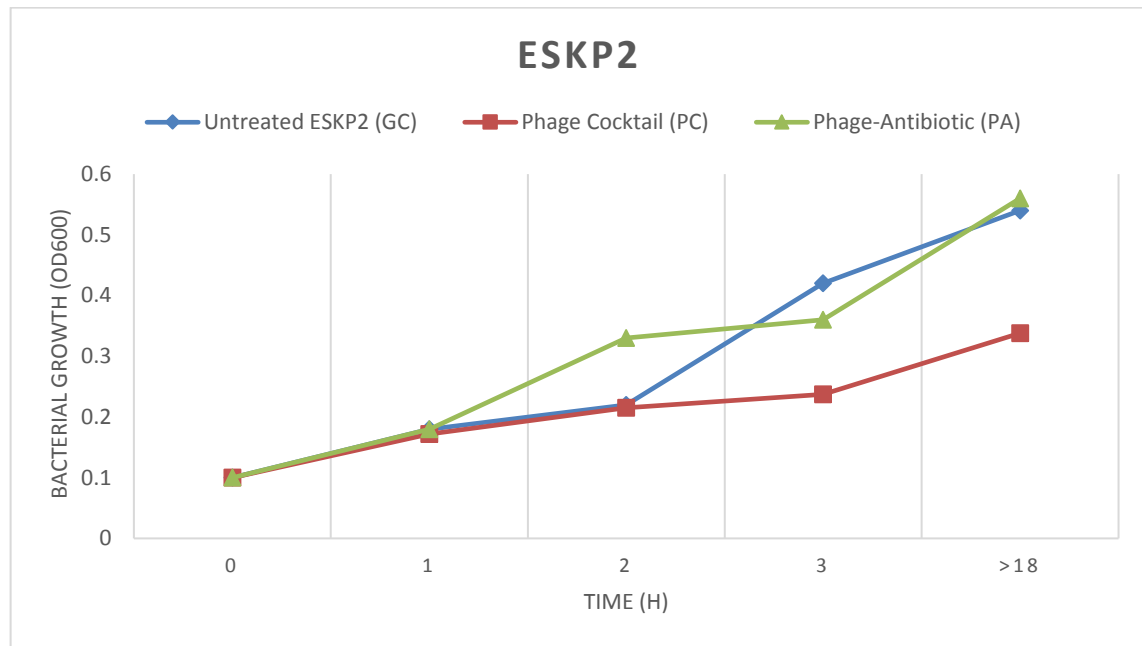


Figure 3.22: Time-kill curves of phage cocktail (PC), phage-antibiotic synergy when treated with sub-inhibitory concentration of Gentamicin (PA) and growth control curve (GC) versus ESKP2.

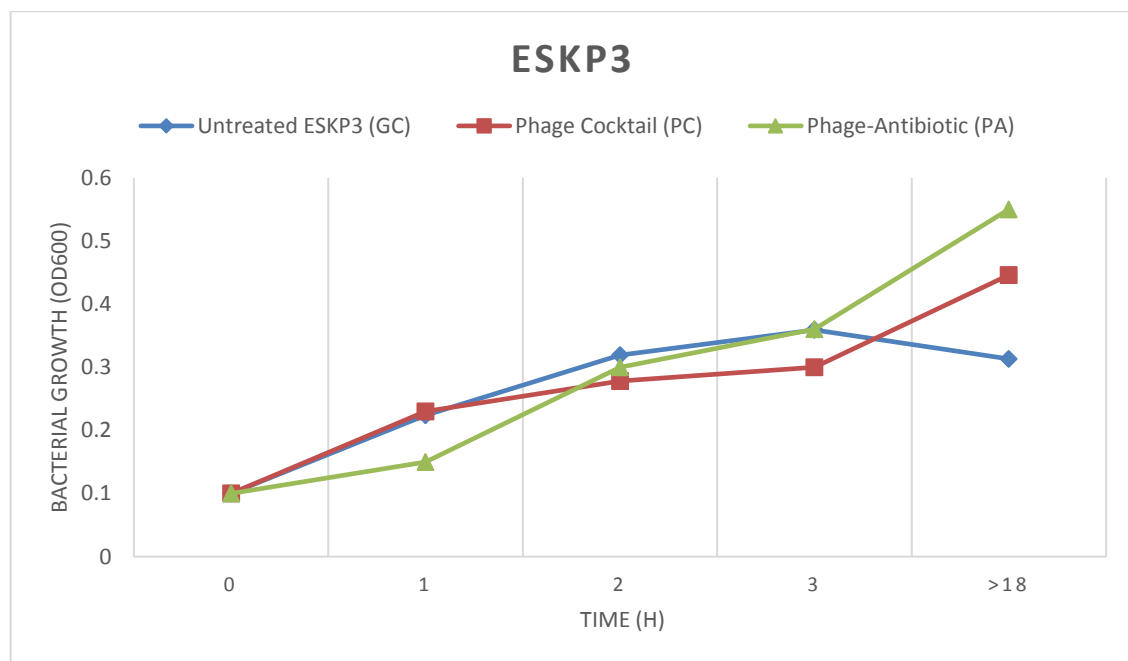


Figure 3.23: Time-kill curves of phage cocktail (PC), phage-antibiotic synergy when treated with sub-inhibitory concentration of Gentamicin (PA) and growth control curve (GC) versus ESKP3.

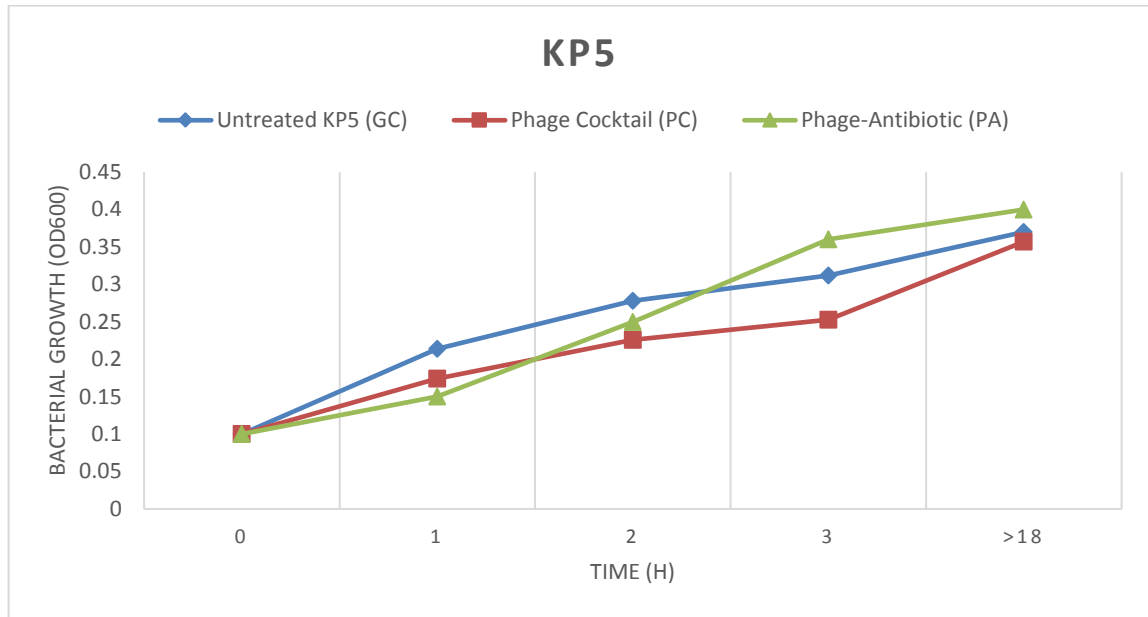


Figure 3.24: Time-kill curves of phage cocktail (PC), phage-antibiotic synergy when treated with sub-inhibitory concentration of Gentamicin (PA) and growth control curve (GC) versus KP5.

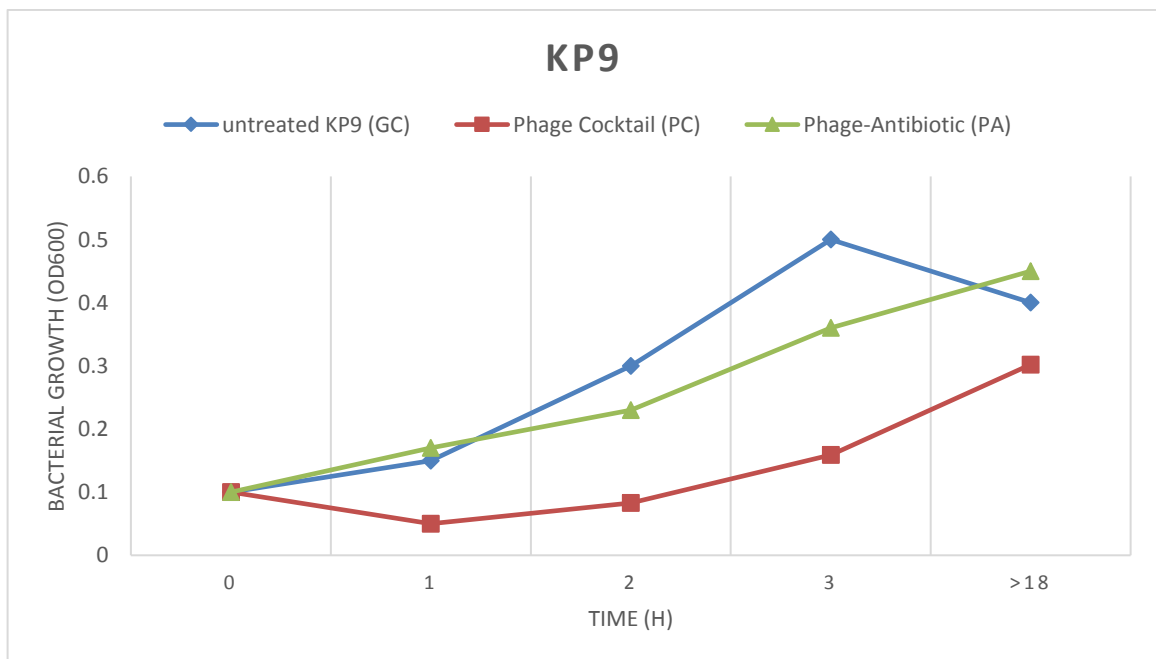


Figure 3.25: Time-kill curves of phage cocktail (PC), phage-antibiotic synergy when treated with a sub-inhibitory concentration of Gentamicin (PA) and growth control curve (GC) versus KP9.

CHAPTER 4

DISCUSSION

4.1 Verification of *Klebsiella pneumoniae*

The vast majority of *Klebsiella* infections, are associated with *Klebsiella pneumoniae*; clinically the most important *Klebsiella* species. The existing methods for detection of this organism include; conventional culture on selective media, biochemical tests, microscopic observation, serological testing etc. and these procedures are found to be time-consuming, less efficient and cumbersome. Therefore, it demands for a speedy diagnostic method for the identification of this opportunistic pathogen for rapid and specific intervention, ultimately to reduce the spread of infectious diseases (Aurna, 2017).

The present study describes the use of a simple PCR based method targeting the 16S rRNA gene for the rapid identification of *K. pneumoniae*. The rRNA genes are ideal candidates for bacterial identification because they are highly conserved within the species. PCR is widely used in many fields of microbiology, such as for direct bacterial detection in clinical specimens or for rapid identification of the cultured colonies. PCR can detect a small number of target organism by amplifying species-specific bacterial genes and bacteria can be identified by nucleotide sequence analysis of the PCR product followed by comparison of this sequence with known sequences stored in a database.

The universal primer set using the PCR protocol described in this study (as to Section 2.6.1) has been able to identify the 16S rRNA region of *K. pneumoniae* directly and specifically in colonies grown on Luria-Bertani (LB) agar plates.

Overall, the target nucleic acid fragments extracted from twenty-one clinical strains were specifically and efficiently amplified using the 16S rRNA gene of *K. pneumoniae* in this present study. DNA extraction from bacterial colonies using Genomic DNA extraction kit is an effective and rapid method (as to Section 2.6.1) used in the microbiology laboratories for the preparation of DNA template. The PCR purified products were subjected to Sanger Sequencing. BLASTn analysis suggested 96-99 % identity with the 16S rRNA gene of *K. pneumoniae* listed in Table 3.1.

4.2 Housekeeping Genes Sequencing Analysis

Besides the usual 16S rRNA gene sequencing, there are other molecular approaches which have been described to identify species of clinically important bacteria (Albuquerque et al. 2009). One of them is by using housekeeping genes sequencing that is often used to develop multi-locus sequence typing (MLST). This scheme is effective in discriminating *K. pneumoniae* which further provides unambiguous data useful for the epidemiology of *K. pneumoniae*.

Diancourt and colleagues (2005) have successfully developed a MLST scheme for *K. pneumoniae* by using sequences of seven housekeeping genes; *rpoB*, *gapA*, *mdh*, *pgi*, *phoE*, *infB* and *tonB*. However, the identification of *K. pneumoniae* strains can generally be confirmed in a simpler way, by selecting only specific housekeeping genes to be sequence analysed (He et al., 2016).

Therefore, in this experiment, only three out of seven housekeeping (as to Table 3.4) were selected due to the fact that the *rpoB* gene, encoding the β -subunit of RNA polymerase, has emerged as a core gene candidate for phylogenetic analyses allowing discrimination of closely related isolates that able to discriminate *Klebsiella* at the species and even subspecies level. Whereas, 16S rRNA gene could only discriminate the clinical isolates at the genus level. This statement is supported by He et al., (2016) that mentioned gene tree-based *rpoB* can be a powerful tool to identify and classify *K. pneumoniae* isolates compare to those based on 16S rRNA genes. The housekeeping gene, *gapA*; an internal positive-control probe was incorporated into the assay in order to confirm DNA template quality and PCR efficiency (Chen et al., 2012).

Capone et al., (2013) have chosen *gapA* and *tonB* genes as the most discriminatory alleles of the multi-locus sequence typing were initially amplified and sequenced to categorise the strains in group of sequence types.

The phylogenetic analysis of all three housekeeping genes; *rpoB*, *gapA* and *tonB* were able to identify KP16 as *Klebsiella quasipneumoniae subsp. similipneumoniae* (ST1484) isolate based on locus sequence query (as to Table 3.4). As shown by the phylogenetic trees in Figure 3.6 to Figure 3.8, KP16 belongs to a different lineage. *K. quasipneumoniae subsp. similipneumoniae* (ST1484) were also found in other countries such as United Kingdom (2002) and Cambodia (2014) with the accession number of ERS1867323 and ERR486312, respectively. A study conducted by Elliott et al., (2016) has supported and confirmed the antibiotic susceptibility testing of *K. quasipneumoniae subsp. similipneumoniae* resistance towards ceftazidime (as to Table 3.3).

4.3 Antibiotic Sensitivity Test

The percentages of *K. pneumoniae* isolates susceptible to the antibiotics that are frequently used to treat *K. pneumoniae* infections are as shown in Table 3.2 and Table 3.3. The clear zones or zones of inhibition around each antibiotic disk indicate the extent of the test organism's ability or inability to survive in the presence of the antibiotic test (Gonzalez-Padilla et al., 2015; Shields et al., 2016).

Table 3.2 and Table 3.3 show *K. pneumoniae* strains were sensitive to only one-third of the tested antibiotics; namely, Amikacin, Cefepime, Imipenem, Meropenem and Gentamicin. However, the strains showed high resistance towards Ampicillin and Trimethoprim / Sulfamethoxazole. Carbapenem-resistant (CRKP1) and ESBL-producing (ESKP2, ESKP3 and ESKP4) *K. pneumoniae* strains showed an alarming indication of multidrug-resistance. The development and spread of antibiotics and multidrug-resistance bacteria showed an increasing trend due to the heavy selective pressure of antibiotics (Amyes & Gemmell, 1992). The high occurrence of ESBL among *K. pneumoniae* is perhaps due to the nature of its nosocomial presence and the survival of diverse replicons carried by the multidrug-resistant *K. pneumoniae* isolates (Mohsen et al., 2016).

Due to the rise of carbapenem resistant and ESBL-producing *K. pneumoniae*, aminoglycosides are proposed to be an alternative and better treatment of *K. pneumoniae* infections (Hirsch & Tam, 2010), since, aminoglycosides are particularly potent against members of the *Enterobacteriaceae* family including *K. pneumoniae* (Krause et al., 2016). Sensitivity of *K. pneumoniae* to Gentamicin, an aminoglycoside could mean that there is a possibility of sensitivity to other aminoglycosides such as Amikacin, Streptomycin, Neomycin and Kanamycin. However, this is not totally certain as each of the

aminoglycosides have a slightly different mechanism of resistance due to their different aminoglycoside modifying enzymes chromosomal mutation e.g. streptomycin and impermeability of membranes (Shields et al., 2016).

4.4 Bacteriophage Isolation

In this study, five novel *Klebsiella* phages were isolated from domestic sewage-samples and were further characterised. Sewage, in general, contains a large diversity of coliforms due to faecal contamination, therefore, is a reservoir of enteric pathogens.

Lytic phages and lysogenic phages can be distinguished from the plaques formed on the agar media, either clear or turbid plaques as shown in Figure 3.9. However, lytic phages are preferred for this study over lysogenic phages, as the latter can lead to the transfer of virulence- and antibiotic-resistance genes (Carlton, 1999) as previously mentioned in Section 1.6.3.

Studies on bacteriophages have revealed that the process is initiated when the virion interacts with host cell surface receptor molecules (Megha et al., 2017). The presence of these zones of clearing indicated that amplifying bacteriophage from a raw sewage sample and inoculating the phage into the host was an effective way of isolating and visualising phage. Amplification of phages in the sewage samples allow them to infect and reproduce within freshly cultured *K. pneumoniae*. The infection spread as the virus replicate and infect nearby cells resulting in more cell lysis and eventually forming clear visible plaques. It is known that most of the bacteriophages have a highly specific receptor that sometimes exhibits either little or no interaction towards receptors with slightly different structure (as to Table 3.6). This specificity forms the basis of numerous phages typing methods for the identification of bacterial species or subspecies. The results obtained as shown in Table 3.6

thus clearly indicated that, the bacteriophages for *K. pneumoniae* were highly specific against its respective host. This suggests that host-specific bacteriophages against human pathogens are prevalent and can be readily isolated from sewage ecosystems (Sundar et al., 2009).

One of the key requirements for developing phage therapy is the ability to quantitate viable bacteriophages accurately and reproducibly, so that rigorous studies of their potencies can be performed (Anderson, 2015).

4.5 Bacteriophages Plaque Morphologies

Morphology of phages is probably one of the most important criteria for characterising phages (Yoon et al., 2007). Variation in the plaque morphology in the present study may correspond to the difference in phage strain (Shende et al., 2017).

The selected phages in this experiment were found to be lytic as shown by the clear zones of lysis on the bacterial lawn (Figure 3.11). The faster the virions diffuse, the faster the plaques enlarge. Two factors impacting phage diffusion are the phage intrinsic properties and agar density (Abedon & Yin, 2009). Under the standardised condition, the plaque morphology can be relatively consistent. Therefore, a change in plaque morphology during the study is commonly used as an indication that the viral strain may have sustained a mutation(s) (Gallet, Kannoly, & Wang, 2011).

4.6 Host Range

Step-by-step method was used to prepare phage cocktail that were able to display broad lytic host range and ensuring that bacteria resistant to one phage remain susceptible to others (Gu et al., 2012). This study has shown that eleven phages isolated through the SBS

method have the ability to lyse all eighteen *K. pneumoniae* strains (refer to Table 3.6). Gu et al., (2012) have concluded that the degree of sensitivity of bacteria towards different phages was caused by different adsorption receptors or the infection mechanism of the phages.

The host range of the isolated phages was determined by spot testing assays on phage-treated bacterial cultures. A positive spot test is indicated when the entire drop area is clear which exhibits the eradication of bacterial growth, whereas the presence of bacterial lawn in the spot region gives a negative spot test (as shown in Figure 3.10). In comparison with phage plaquing, spot testing can be employed as a simple and rapid initial screen to determine which bacterial strains are outside a phage's bactericidal host range (Hyman & Abedon, 2010).

The specificity of bacteriophages against their bacterial host was validated when the growth of bacteria was significantly reduced either on solid agar as well as in broth culture. In addition, these phages have been found to remain viable even after 3 to 4 weeks of isolation, when stored at 4 °C. This provides a great opportunity for the long-lasting phages to be used for the prophylaxis of bacterial infections as well as for the treatment of biofilms (Sundar et al., 2009).

4.7 Single-Step Phage Growth Curve

As shown in Figure 3.12 to Figure 3.16, the selected phages showed a relatively large burst size with a short latent period, which is further suggestive of the lytic nature of the phage. Except for ϕ KPaV04 which showed a slightly longer latent period of 60 mins. Several factors such as ions (e.g., Mg^{2+} and Ca^{2+}), pH, temperature, and the growth medium can influence phage adsorption rate and its infection. Previously, it has been reported that calcium and magnesium ions have contributed a positive effect on the phage-bacterium

attachment. Therefore, in this study, 10 mM of Calcium Chloride (CaCl_2) concentration were added to the phage growth medium because these ions have an electrostatic bonding effect on the interactions of phage bacterium systems (Li & Zhang, 2014). Calcium ions stabilize the interaction of virion with receptors. Different concentrations of calcium ions have shown maximum effects on infectivity of different phages.

4.8 Bacteriophage Structural Proteins Analysis

The aim of studying phage structural proteins is to investigate the diversity of phage in different environments (Adriaenssens & Cowan, 2014). Unlike 16S rRNA in Bacteria and Archaea, prokaryotic or eukaryotic viruses lack the universal signature gene. Therefore, many different genes were assessed as potential group-specific signature genes such as portal protein (g20) and major capsid protein (g23).

Portal protein (g20) and major capsid protein (g23) specifically target cyanophages and T4-related members of Myoviridae, respectively. However, amplification of g20 genes in all five selected phages showed no visible bands during electrophoresis. It can be deduced that the isolated phages do not belong to the cyanophages family. On the other hand, major capsid protein (g23) is highly conserved among viruses and is frequently used for taxonomical and phylogenic classifications (Bamford et al., 2005). Amplification of g23 gene managed to produce distinct bands in ϕKPaV08 and ϕKPaV12 (as to Figure 3.18). The g23 gene appears to span a much greater diversity of bacteriophage groups within the Myoviridae family than the g20 gene, encompassing both cyanophages and non-cyanophages and making it a more desirable marker gene for investigating myo-virus diversity (Adriaenssens & Cowan, 2014).

Phage enzymes (lysins) have the ability to break covalent bonds of peptidoglycan/murein layers of the host cell wall, causing the release of newly assembled phage progenies (Fischetti, 2005). Endolysin has a unique ability to lyse the murein layer of the cell wall of the host cell in a species-specific manner. Therefore, endolysins have the potential to be used as a novel class of therapeutic agents without any side or toxic effects (Mishra et al., 2014). Clear band produced by ϕ KPaV03 and ϕ KPaV08 lysin gene (as to Figure 3.17) has justified the reason behind their broad host range with the ability to lyse more than five *K. pneumoniae* strains (as to Section 3.5.3).

As for tail fiber genes, all selected phages managed to produce visible bands with phages ϕ KPaV03 and ϕ KPaV12 exhibiting multiple bands of different sizes (as to Figure 3.19). Therefore, the tail fiber gene of these two phages was not selected for further analysis. Often tail bacteriophages constitute the order of *Caudovirales*, which is divided into 3 families: *Myoviridae*, *Siphoviridae* and *Podoviridae*. Hence, tail fiber genes can help distinguish phages among the 3 families.

4.9 Minimum Inhibitory Concentration (MIC)

The aim of broth dilution methods is to determine the minimal concentration of the assayed antimicrobial agent inhibiting the visible growth of the target bacterium under specific test conditions. MIC values are used to determine the susceptibilities of bacteria to drugs and to evaluate the activity of new antimicrobial agents.

In this experiment, MIC broth dilution was performed due to the limitation of Antibiotic Sensitivity Test (Kirby-Bauer disc). AST was done on agar with the limited range of antibiotic concentration supplied by the manufacturer. Therefore, MIC broth dilution was performed to validate *K. pneumoniae* strains that were resistant or showed intermediate

result against Gentamicin (as to Table 3.11). The MIC analysis confirmed that five resistant strains mentioned from the previous section were resistant to Gentamicin having higher MIC than 2 µg / mL.

4.10 Phage Cocktail

Formulation of phages into cocktail increases their potential to be used presumptively, that is, prior to the identification of pathogens in order to determine the phage susceptibility. Additionally, when more phages are included to the cocktail, the greater the potential that there will be sustainable levels of medical as well as commercial demand for a given formulation (Chan et al., 2013).

The phage titer is also important for reaching a productive infection during phage therapy, but the titer of individual phages in a phage cocktail would get lower and lower as more phages are added. A phage with a narrow host range and/or low efficacy must thus be discarded when assembling a phage cocktail (Mirzaei & Nilsson, 2015). Therefore, five selected bacteriophages with broad host range and high titer were added each with equal volume of MOI 1 to produce a phage cocktail.

Based on Figure 3.20, *K. aerogenes* (KA2) functioned as a positive control to determine the infection of phage cocktail against other *Klebsiella* spp. Significant decrease of *K. aerogenes* (KA2) growth after 18 h has proven that phage cocktail was not only able to infect *K. pneumoniae* but also different *Klebsiella* species. Therefore, optimisation of the phage cocktail formulation will make it possible to extend the spectrum of bacterial hosts infection.

4.11 Phage-Antibiotic Synergy (PAS) Approaches

As previously stated in Section 2.11.3, *Klebsiella pneumoniae* strains subjected to phage-antibiotic synergy analysis were those resistant towards antibiotic Gentamicin. Three out of the five resistant strains belonged to either carbapenem-resistant *K. pneumoniae* (CRKP) or extended-spectrum beta-lactamase (ESBL) strains (as to Table 3.1). Antibiotic Gentamicin is classified under Aminoglycosides, known to be potent and broad-spectrum that act through inhibition of protein synthesis (Krause et al., 2016).

Phage-antibiotic synergism (PAS) was performed to determine if the combination of multiple agents improves killing. PAS has been formerly reported to be a possible approach against *Pseudomonas aeruginosa*, *Escherichia coli* (Knezevic et al., 2013) and *Burkholderia cepacia* (Kamal & Dennis, 2015). The mechanism of PAS is proposed to result from antibiotic-induced bacterial elongation, which may facilitate the access of phages to their bacterial target (Oechslin, 2017). According to Comeau et al., (2007), phage-antibiotic synergism is a result of a change in morphology that permits faster assembly of phages through altered or larger pools of precursors that leads to phage maturation and accelerates the timing of cell lysis.

From previous Section 2.11.2, the MIC value for antibiotics have been determined and sub-inhibitory concentration was selected to study the growth Gentamicin-resistant *K. pneumoniae* strains in combination with phage cocktail. In Section 2.11.3, *K. pneumoniae* ATCC KP (Gentamicin sensitive) was used as a positive control to observe the PAS trend. CRKP1 showed the synergistic effect of phage cocktail with sub-inhibitory antibiotics. It can be observed that PAS was able to reduce the bacterial growth at a certain hour, even lower than phage cocktail shown in Figure 3.21, and Figure 3.22. However, it is best to note

that the efficacy of some antibiotics for treating bacterial infections can be considerably augmented when the phages were administered before the antibiotic rather than if they are simultaneously administered (Chaudhry et al., 2017). This method will greatly improve future results of Phage-Antibiotic Synergy (PAS) approach.

CHAPTER 5

CONCLUSION AND FUTURE STUDIES

In conclusion, five newly isolated *Klebsiella pneumoniae* bacteriophages from domestic sewage, namely, ϕ KPaV03, ϕ KPaV04, ϕ KPaV08, ϕ KPaV10 and ϕ KPaV12 have shown to exhibit satisfactory preliminary characterisation. Selection of these bacteriophages were based on their significant lytic properties by having good plaquing ability on both clinical- and community-acquired *Klebsiella* isolates. Besides that, these selected phages displayed efficient growth characteristics by having high titer and possessing a broad host range.

They were further investigated to determine their efficiency in infecting *K. pneumoniae* as a phage cocktail as well as through phage-antibiotic synergy approach. The result showed that the phage cocktail was more effective than phage-antibiotic synergy in reducing Gentamicin-resistant *K. pneumoniae*. However, phage-antibiotic synergy has shown to produce better results than phage cocktail when infecting *K. pneumoniae* with either sensitive or intermediate reaction towards Gentamicin.

Moreover, the use of subinhibitory concentrations of antibiotics results in avoidance of antibiotic side effects occurring after administration of high antibiotic dosage. Therefore, these isolated phages can not only function as a mono-phage but also be applied either in a phage cocktail or synergism of phage with antibiotic; which they have great therapeutic potential for treating wide-range of *K. pneumoniae* infections including multi-drug resistant infections.

Hence, for better characterisation and understanding of the isolated *K. pneumoniae* bacteriophages, whole-genome sequencing can be conducted in the upcoming study. Future studies should also take in consideration in investigating the optimum dosage or concentration of phage cocktail and synergism of phage-antibiotic (PAS) when infecting multi-drug resistant bacterial infections. Furthermore, to better understand the mechanisms of rapid synergism, the observed phenomenon should be assessed for possible applications in control of *K. pneumoniae*.

REFERENCES

- Abedon, S. T., & Yin, J. (2009). Bacteriophage Plaques: Theory and Analysis. *Methods in Molecular Biology Bacteriophages*, 501, 161-174. doi:10.1007/978-1-60327-164-6_17
- Abubakar, S., Suleiman, B. H., Abbagana, B. A., Mustafa, I. A., & Musa, I. A. (2016). Novel Uses of Bacteriophages in the Treatment of Human Infections and Antibiotic Resistance. *American Journal of BioScience*, 4(3), 34-40. doi:10.11648/j.ajbio.20160403.13
- Ackermann, H. W. (2011). Bacteriophage Taxonomy. *Microbiology Australia*, 32, 90-94
- Adriaenssens, E. M., & Cowan, D. A. (2014). Using signature genes as tools to assess environmental viral ecology and diversity. *Applied and Environmental Microbiology*, 80(15), 4470–4480. <http://doi.org/10.1128/AEM.00878-14>
- Akinkunmi, E. O., Adesunkanmi, A., & Lamikanra, A. (2014). Pattern of pathogens from surgical wound infections in a Nigerian hospital and their antimicrobial susceptibility profiles. *African Health Sciences*, 14(4), 802-809. doi:10.4314/ahs.v14i4.5
- Al-Marzooq, F., Yusof, M. Y., & Tay, S. T. (2015). Molecular Analysis of Antibiotic Resistance Determinants and Plasmids in Malaysian Isolates of Multidrug Resistant *Klebsiella pneumoniae*. *PloS One*, 10(7), 1-21. doi:10.1371/journal.pone.0133654
- Aurna, S. T. (2017). Rapid identification of *Klebsiella pneumoniae* using PCR based method targeting 16S rRNA gene. Dissertation submitted to BRAC University, Dhaka, Bangladesh. <http://hdl.handle.net/10361/9163>
- Barrangou, R., & Horvath, P. (2009). The CRISPR system protects microbes against phages,

- plasmids. *Microbe*, 4(5), 224–230. <https://doi.org/10.1128/microbe.4.224.1>
- Bradley, D. E. (1967). Ultrastructure of bacteriophage and bacteriocins. *Bacteriological Reviews*, 31(4), 230–314.
- Brüssow, H. (2013). Bacteriophage-host interaction: From splendid isolation into a messy reality. *Current Opinion in Microbiology*, 16(4), 500–506. <http://doi.org/10.1016/j.mib.2013.04.007>
- Bull, J. J., Levin, B. R., DeRouin, T., Walker, N., & Bloch, C. A. (2002). Dynamics of success and failure in phage and antibiotic therapy in experimental infections. *BMC Microbiology*, 2(35). doi:10.1186/1471-2180-2-35
- Bush, K. (2010). Alarming β -lactamase-mediated resistance in multidrug-resistant *Enterobacteriaceae*. *Current Opinion in Microbiology*, 13(5), 558–564. <http://doi.org/10.1016/j.mib.2010.09.006>
- Bush, K., Jacoby, G. A., & Medeiros, A. A. (1995). A functional classification scheme for β -lactamases and its correlation with molecular structure. *Antimicrobial Agents and Chemotherapy*, 39(6), 1211–1233. <http://doi.org/10.1128/AAC.39.6.1211>
- Cao, F., Wang, X., Wang, L., Li, Z., Che, J., Wang, L., . . . Xu, Y. (2015). Evaluation of the Efficacy of a Bacteriophage in the Treatment of Pneumonia Induced by Multidrug Resistance *Klebsiella pneumoniae* in Mice. *BioMed Research International*, 2015, 1-9. doi:10.1155/2015/752930
- Capone, A., Giannella M., Fortini, D., Giordano, A., Meledandri, M., Ballardini, M., . . . Petrosillo, N. (2013). High rate of colistin resistance among patients with carbapenem-resistant *Klebsiella pneumoniae* infection accounts for an excess of mortality. *Clinical*

Microbiology and Infection, 19(1), E23–E30. doi: 10.1111/1469-0691.12070

- Carlton, R. M. (1999). Phage therapy: past history and future prospects. *Archivum Immunologiae et Therapiae Experimentalis*, 47(5), 267–274. <http://doi.org/10.2217/fvl.15.3>
- Chadha, P., Katare, O. P., & Chhibber, S. (2016). In vivo efficacy of single phage versus phage cocktail in resolving burn wound infection in BALB/c mice. *Microbial Pathogenesis*, 99, 68–77. <http://doi.org/10.1016/j.micpath.2016.08.001>
- Chan, B. K., Abedon, S. T., & Loc-Carrillo, C. (2013). Phage cocktails and the future of phage therapy. *Future Microbiology*, 8(6), 769–783. doi:10.2217/fmb.13.47
- Chang, H. C., Chen, C. R., Lin, J. W., Shen, G. H., Chang, K. M., Tseng, Y. H., & Weng, S. F. (2005). Isolation and characterization of novel giant *Stenotrophomonas maltophilia* phage phiSMA5. *Applied and Environmental Microbiology*, 71(3), 1387–1393. doi:10.1128/AEM.71.3.1387-1393.2005
- Chang, V. S., Dhaliwal, D. K., Raju, L., & Kowalski, R. P. (2015). Antibiotic Resistance in the Treatment of *Staphylococcus aureus* Keratitis: A 20-Year Review. *Cornea*, 34(6), 689–703. doi:10.1097/ICO.0000000000000431
- Chaturongakul, S., & Ounjai, P. (2014). Phage-host interplay: examples from tailed phages and Gram-negative bacterial pathogens. *Frontiers in Microbiology*, 5, 442. doi:10.3389/fmicb.2014.00442
- Chaudhry, W. N., Concepción-Acevedo, J., Park, T., Andleeb, S., Bull, J. J., & Levin, B. R. (2017). Synergy and Order Effects of Antibiotics and Phages in Killing *Pseudomonas aeruginosa* Biofilms. *PloS One*, 12(1), e0168615.

doi:<https://doi.org/10.1371/journal.pone.0168615>

- Chen, L., Chavda, K. D., Mediavilla, J. R., Zhao, Y., Fraimow, H. S., Jenkins, S. G., ... Kreiswirth, B. N. (2012). Multiplex real-time PCR for detection of an epidemic KPC-producing *Klebsiella pneumoniae* ST258 clone. *Antimicrobial Agents and Chemotherapy*, 56(6), 3444–3447. <http://doi.org/10.1128/AAC.00316-12>
- Chung, P. Y. (2016). The emerging problems of *Klebsiella pneumoniae* infections: Carbapenem resistance and biofilm formation. *FEMS Microbiology Letters*, 363(20), 1–6. <http://doi.org/10.1093/femsle/fnw219>
- Cisek, A. A., Dąbrowska, I., Gregorczyk, K. P., & Wyżewski, Z. (2017). Phage Therapy in Bacterial Infections Treatment: One Hundred Years After the Discovery of Bacteriophages. *Current Microbiology*, 74(2), 277–283. <http://doi.org/10.1007/s00284-016-1166-x>
- Clegg, S., & Murphy, C. N. (2016). Epidemiology and Virulence of *Klebsiella pneumoniae*. *Microbiology Spectrum*, 4(1), 1-17. doi:10.1128/microbiolspec.uti-0005-2012
- Comeau, A. M., Tétart, F., Trojet, S. N., Prère, M. F., & Krisch, H. M. (2007). Phage-antibiotic synergy (PAS): β -lactam and quinolone antibiotics stimulate virulent phage growth. *PLoS One*, 2(8), 8–11. <http://doi.org/10.1371/journal.pone.0000799>
- Demeke, A., Yibrah, T., & Ashlew, M. (2015). Bacteriophage therapy against antibiotic resistant bacteria. *Direct Research Journal of Agriculture and Food Science*, 3(10), 184-192. doi:10.26765/DRJAFS
- Delbruck, M. (1940). the Growth of Bacteriophage and Lysis of the Host. *The Journal of General Physiology*, 23(5), 643–660. <http://doi.org/10.1085/jgp.23.5.643>

- Deveau, H., Barrangou, R., Garneau, J. E., Labonté, J., Fremaux, C., Boyaval, P., ... Moineau, S. (2008). Phage response to CRISPR-encoded resistance in *Streptococcus thermophilus*. *Journal of Bacteriology*, 190(4), 1390–1400. <https://doi.org/10.1128/JB.01412-07>
- Diancourt, L., Passet, V., Verhoef, J., Grimont, P. A., & Brisse, S. (2005). Multilocus Sequence Typing of *Klebsiella pneumoniae* Nosocomial Isolates. *Journal of Clinical Microbiology*, 43(8), 4178-4182. doi:10.1128/JCM.43.8.4178-4182.2005
- Drulis-Kawa, Z., Mackiewicz, P., Kęsik-Szeloch, A., Maciaszczyk-Dziubinska, E., Weber-Dąbrowska, B., Dorotkiewicz-Jach, A., . . . Kropinski, A. M. (2011). Isolation and characterisation of KP34—a novel φKMV-like bacteriophage for *Klebsiella pneumoniae*. *Applied Microbiology and Biotechnology*, 90(4), 1333-1345. doi:10.1007/s00253-011-3149-y
- Dublanchet, A., & Bourne, S. (2007). The epic of phage therapy. *Canadian Journal of Infectious Diseases and Medical Microbiology*, 18(1), 15–18. <http://doi.org/10.1155/2007/365761>
- El-Shibiny, A., & El-Sahhar, S. (2017). Bacteriophages: The possible solution to treat infections caused by pathogenic bacteria. *Canadian Journal of Microbiology*, 63(11), 865-879. doi:10.1139/cjm-2017-0030
- Elbreki, M., Ross, R. P., Hill, C., Omahony, J., McAuliffe, O., & Coffey, A. (2014). Bacteriophages and Their Derivatives as Biotherapeutic Agents in Disease Prevention and Treatment. *Journal of Viruses*, 2014, 1-20. doi:10.1155/2014/382539
- Elliott, A. G., Ganesamoorthy, D., Coin, L., Cooper, M. A., & Cao, M. D. (2016). Complete

- Genome Sequence of *Klebsiella quasipneumoniae* subsp. *similipneumoniae* Strain ATCC 700603. *Genome Announcements*, 4(3), e00438-16. doi:10.1128/genomeA.00438-16
- Ellison, C. K., Dalia, T. N., Vidal Ceballos, A., Wang, J. C. Y., Biais, N., Brun, Y. V., & Dalia, A. B. (2018). Retraction of DNA-bound type IV competence pili initiates DNA uptake during natural transformation in *Vibrio cholerae*. *Nature Microbiology*, 3(7), 773–780. <http://doi.org/10.1038/s41564-018-0174-y>
- Eriksson, H. (2015). *Bacterial viruses targeting multi-resistant Klebsiella pneumoniae and Escherichia coli*. Stockholm: Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University. ISBN 978-91-7649-123-2
- Fair, R. J., & Tor, Y. (2014). Antibiotics and Bacterial Resistance in the 21st Century. *Perspectives in Medicinal Chemistry*, 6, 25-64. doi:10.4137/pmc.s14459
- Fischetti, V. A. (2005). Bacteriophage lytic enzymes: novel anti-infectives, *Trends in Microbiology*, 13(10): 491-496. <http://doi.org/10.1016/j.tim.2005.08.007>
- Gallet, R., Kannoly, S., & Wang, I. (2011). Effects of bacteriophage traits on plaque formation. *BMC Microbiology*, 11(1), 181-197. <http://doi.org/10.1186/1471-2180-11-181>
- Gonzalez-Padilla, M., Torre-Cisneros, J., Rivera-Espinar, F., Pontes-Moreno, A., López-Cerero, L., Pascual, A., ... Rodríguez-Baño, J. (2015). Gentamicin therapy for sepsis due to carbapenem-resistant and colistin-resistant *Klebsiella pneumoniae*. *Journal of Antimicrobial Chemotherapy*, 70(3), 905–913. <http://doi.org/10.1093/jac/dku432>
- Gu, J., Liu, X., Li, Y., Han, W., Lei, L., Yang, Y., . . . Feng, X. (2012). A Method for

- Generation Phage Cocktail with Great Therapeutic Potential. *PLoS One*, 7(3). doi:10.1371/journal.pone.0031698
- Harper, D. R., & Enright, M. C. (2011). Bacteriophages for the treatment of *Pseudomonas aeruginosa* infections. *Journal of Applied Microbiology*, 111(1), 1–7. <http://doi.org/10.1111/j.1365-2672.2011.05003.x>
- He, Y., Guo, X., Xiang, S., Li, J., Li, X., Xiang, H., ... Chen, J. (2016). Comparative analyses of phenotypic methods and 16S rRNA, *khe*, *rpoB* genes sequencing for identification of clinical isolates of *Klebsiella pneumoniae*. *Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology*, 109(7), 1029–1040. <http://doi.org/10.1007/s10482-016-0702-9>
- Ho, Karen. (2001). Bacteriophage Therapy for Bacterial Infections: Rekindling a Memory from the Pre-Antibiotics Era. *Perspectives in Biology and Medicine*, 44(1), 0–16. <http://doi.org/10.1353/pbm.2001.0006>
- Holmes, R. (2000). Biology and Molecular Epidemiology of Diphtheria Toxin and the Tox Gene. *The Journal of Infectious Diseases*, 181(S1), 156-167. doi:10.1086/315554
- Holt, K. E., Wertheim, H., Zadoks, R. N., Baker, S., Whitehouse, C. A., Dance, D., . . . Thomson, N. R. (2015). Genomic analysis of diversity, population structure, virulence, and antimicrobial resistance in *Klebsiella pneumoniae*, an urgent threat to public health. *Proceedings of the National Academy of Sciences*, 112(27), 3574-3581. doi:10.1073/pnas.1501049112
- Hoyles, L., Murphy, J., Neve, H., Heller, K. J., Turton, J. F., Mahony, J., . . . Sinderen, D. V. (2015). *Klebsiella pneumoniae subsp.pneumoniae*–bacteriophage combination from

- the caecal effluent of a healthy woman. *PeerJ*, 3, e1061. doi:10.7717/peerj.1061
- Hyman, P., & Abedon, S. T. (2010). Bacteriophage Host Range and Bacterial Resistance. *Advances in Applied Microbiology*, 70, 217-248. doi:10.1016/s0065-2164(10)70007-1
- Jeney, F. (2012). Controversy in Virology: Bacteriophage Therapy versus Antibiotics. *The First Year Papers (2010 - Present)*, 33, 1-5.
- Jing, R., Liu, J., Yu, Z., Liu, X., & Wang, G. (2014). Phylogenetic distribution of the capsid assembly protein gene (g20) of cyanophages in paddy floodwaters in Northeast China. *PLoS One*, 9(2): e88634. <http://doi.org/10.1371/journal.pone.0088634>
- Karumidze, N., Kusradze, I., Rigvava, S., Goderdzishvili, M., Rajakumar, K., & Alavidze, Z. (2012). Isolation and characterisation of lytic bacteriophages of *Klebsiella pneumoniae* and *Klebsiella oxytoca*. *Current Microbiology*, 66(3), 251–258. <http://doi.org/10.1007/s00284-012-0264-7>
- Kaur, R., Matta, T., & Kaur, H. (2017). Transhumance and protected areas in West Africa: State of play and Management mechanisms review. *Haya: The Saudi Journal of Life Sciences*, 2(8), 269–283. <http://doi.org/10.21276/haya>
- Khater, F., Balestrino, D., Charbonnel, N., Dufayard, J. F., Brisse, S., & Forestier, C. (2015). In Silico Analysis of Usher Encoding Genes in *Klebsiella pneumoniae* and Characterization of Their Role in Adhesion and Colonization. *PloS One*, 10(3), 1-24. doi:10.1371/journal.pone.0116215
- Kidd, T. J., Mills, G., Sá-Pessoa, J., Dumigan, A., Frank, C. G., Insua, J. L., ... Bengoechea, J. A. (2017). A *Klebsiella pneumoniae* antibiotic resistance mechanism that subdues host defences and promotes virulence. *EMBO Molecular Medicine*, 9(4), 430–447.

<http://doi.org/10.15252/emmm.201607336>

- Koskella, B., & Meaden, S. (2013). Understanding bacteriophage specificity in natural microbial communities. *Viruses*, 5(3), 806–823. <http://doi.org/10.3390/v5030806>
- Krause, K. M., Serio, A. W., Kane, T. R., & Connolly, L. E. (2016). Aminoglycosides: An overview. *Cold Spring Harbor Perspectives in Medicine*, 6(6), 1–18. <http://doi.org/10.1101/cshperspect.a027029>
- Kumari, S., Harjai, K., & Chhibber, S. (2010). Isolation and characterization of *Klebsiella pneumoniae* specific bacteriophages from sewage samples. *Folia Microbiologica*, 55(3), 221-227. doi:10.1007/s12223-010-0032-7
- Kutter, E. & Sulakvelidze, A. (2005). *Bacteriophages Biology and Applications*, 1st Edition, CRC Press Book. ISBN 9780849313363.
- Landsberger, M., Gandon, S., Meaden, S., Rollie, C., Chevallereau, A., Chabas, H., ... van Houte, S. (2018). Anti-CRISPR Phages Cooperate to Overcome CRISPR-Cas Immunity. *Cell*, 174(4), 908-916.e12. <https://doi.org/10.1016/j.cell.2018.05.058>
- Lean, S.-S., Suhaili, Z., Ismail, S., Rahman, N. I. A., Othman, N., Abdullah, F. H., ... Thong, K.-L. (2014). Prevalence and Genetic Characterization of Carbapenem- and Polymyxin-Resistant *Acinetobacter baumannii* Isolated from a Tertiary Hospital in Terengganu, Malaysia. *ISRN Microbiology*, 2014, 1–9. <http://doi.org/10.1155/2014/953417>
- Levy, S. B., & Bonnie, M. (2004). Antibacterial resistance worldwide: Causes, challenges and responses. *Nature Medicine*, 10(12S), S122–S129. <http://doi.org/10.1038/nm1145>

- Li, B., Zhao, Y., Liu, C., Chen, Z., & Zhou, D. (2014). Molecular pathogenesis of *Klebsiella pneumoniae*. *Future Microbiology*, 9(9), 1071-1081. doi:10.2217/fmb.14.48.
- Li, L., & Zhang, Z. (2014). Isolation and characterization of a virulent bacteriophage SPW specific for *Staphylococcus aureus* isolated from bovine mastitis of lactating dairy cattle. *Molecular Biology Reports*, 41(9), 5829-5838. doi:10.1007/s11033-014-3457-2
- Lim, K. T., Yeo, C. C., Yasin, R. M., Balan, G., & Thong, K. L. (2009). Characterization of multidrug-resistant and extended-spectrum-lactamase-producing *Klebsiella pneumoniae* strains from Malaysian hospitals. *Journal of Medical Microbiology*, 58(11), 1463-1469. doi:10.1099/jmm.0.011114-0
- Lin, D. M., Koskella, B., & Lin, H. C. (2017). Phage therapy: An alternative to antibiotics in the age of multi-drug resistance. *World Journal of Gastrointestinal Pharmacology and Therapeutics*, 8(3), 162-173. <http://doi.org/10.4292/wjgpt.v8.i3.162>
- Lin, M.-F., Lin, Y.-Y., & Lan, C.-Y. (2014). Minimal Inhibitory Concentration (MIC) Assay for *Acinetobacter baumannii*. *Bio-Protocol*, 4(23), e1308. <http://doi.org/10.21769/BioProtoc.1308>
- Loc-Carrillo, C., & Abedon, S. (2011). Pros and cons of phage therapy. *Bacteriophage*, 1(2), 111–114. <http://doi.org/10.4161/bact.1.2.14590>
- Loh, L. C., Chin, H. K., Chong, Y. Y., Jeyaratnam, A., Raman, S., Vijayasingham, P., ... Kumar, S. (2007). *Klebsiella pneumoniae* respiratory isolates from 2000 to 2004 in a Malaysian hospital: Characteristics and relation to hospital antibiotics consumption. *Singapore Medical Journal*, 48(9), 813–818.
- Low, Y., Yap, P. S., Jabar, K. A., Ponnampalavanar, S., Karunakaran, R., Velayuthan, R., .

- .. Teh, C. S. (2017). The emergence of carbapenem resistant *Klebsiella pneumoniae* in Malaysia: Correlation between microbiological trends with host characteristics and clinical factors. *Antimicrobial Resistance & Infection Control*, 6(1), 1-13. doi:10.1186/s13756-016-0164-x
- Mahdi Yahya Mohsen, S., Hamzah, H. A., Muhammad Imad Al-Deen, M., & Baharudin, R. (2016). Antimicrobial susceptibility of *Klebsiella pneumoniae* and *Escherichia coli* with extended-spectrum β -lactamase associated genes in hospital Tengku Ampuan Afzan, Kuantan, Pahang. *Malaysian Journal of Medical Sciences*, 23(2), 14–20.
- Matsuzaki, S., Rashel, M., Uchiyama, J., Sakurai, S., Ujihara, T., Kuroda, M., ... Imai, S. (2005). Bacteriophage therapy: A revitalized therapy against bacterial infectious diseases. *Journal of Infection and Chemotherapy*, 11(5), 211–219. <http://doi.org/10.1007/s10156-005-0408-9>
- Megha, P. U., Murugan, S., & Harikumar, P. S. (2017). Isolation and Characterization of Lytic Coliphages from Sewage Water. *Journal of Pure and Applied Microbiology*, 11(1), 559-565.
- Merril, C. R., Scholl, D., & Adhya, S. L. (2003). The prospect for bacteriophage therapy in Western medicine. *Nature Reviews Drug Discovery*, 2(6), 489–497. <http://doi.org/10.1038/nrd1111>
- Mirzaei, M. K. (2016). *The efficacy of bacterial viruses against multi-resistant Escherichia coli: from isolation to pharmacology* (PhD dissertation). Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University, Stockholm. Retrieved from <http://urn.kb.se/resolve?urn=urn:nbn:se:su:diva-126328>

- Mirzaei, M. K., & Nilsson, A. S. (2015). Isolation of Phages for Phage Therapy: A Comparison of Spot Tests and Efficiency of Plating Analyses for Determination of Host Range and Efficacy. *PloS One*, *10*(3), 1-13. doi:10.1371/journal.pone.0118557
- Mishra, A. K., Sharma, N., Kumar, A., Kumar, N., Gundallahalli Bayyappa, M. R., Kumar, S., & Kumar, N. (2014). Isolation, characterization and therapeutic potential assessment of bacteriophages virulent to *Staphylococcus aureus* associated with goat mastitis. *Iranian Journal of Veterinary Research*, *15*(4), 320–325.
- Navidinia, M. (2016). The clinical importance of emerging ESKAPE pathogens in nosocomial infections. *Journal of Paramedical Sciences*, *7*(3), 43-57. doi:10.22037/jps.v7i3.12584
- Nilsson, A. S. (2014). Phage therapy-constraints and possibilities. *Upsala Journal of Medical Sciences*, *119*(2), 192–198. <http://doi.org/10.3109/03009734.2014.902878>
- Podschun, R., & Ullmann, U. (1998). *Klebsiella* spp. as Nosocomial Pathogens: Epidemiology, Taxonomy, Typing Methods, and Pathogenicity Factors. *Clinical Microbiology Reviews*, *11*(4), 589-603. doi:10.1128/cmr.11.4.589
- Rhoads, D. D., Wolcott, R. D., Kuskowski, M. A., Wolcott, B. M., Ward, L. S., & Sulakvelidze, A. (2009). Bacteriophage therapy of venous leg ulcers in humans: Results of a phase I safety trial. *Journal of Wound Care*, *18*(6), 240-243. doi:10.12968/jowc.2009.18.6.42801
- Robert, T. S., Biswas, B., Gill, J. J., Hernandez-Morales, A., Lancaster, J., Lessor, L., . . . Hamilton, T. (2017). Development and Use of Personalized Bacteriophage-Based Therapeutic Cocktails To Treat a Patient with a Disseminated Resistant *Acinetobacter*

- baumannii* Infection. *Antimicrobial Agents and Chemotherapy*, 61(10). doi:10.1128/aac.00954-17
- Rosamond, J., & Allsop, A. (2000). Harnessing the Power of the Genome in the Search for New Antibiotics. *Science*, 287(5460), 1973-1976. doi:10.1126/science.287.5460.1973
- Sambrook, J., Fritsch, E. F., & Maniatis, T. (1989). *Molecular cloning : A laboratory manual* (2nd ed.). New York: Cold Spring Harbor Laboratory Press.
- Santajit, S., & Indrawattana, N. (2016). Mechanisms of Antimicrobial Resistance in ESKAPE Pathogens. *BioMed Research International*, 2016, 1-8. doi:10.1155/2016/2475067
- Sarker, S. A., Mccallin, S., Barretto, C., Berger, B., Pittet, A., Sultana, S., . . . Brüssow, H. (2012). Oral T4-like phage cocktail application to healthy adult volunteers from Bangladesh. *Virology*, 434(2), 222-232. doi:10.1016/j.virol.2012.09.002
- Shende, R. K., Hirpurkar, S. D., Sannat, C., Rawat, N., & Pandey, V. (2017). Isolation and characterization of bacteriophages with lytic activity against common bacterial pathogens. *Veterinary World*, 10(8), 973-978. doi:10.14202/vetworld.2017.973-978
- Shields, R. K., Clancy, C. J., Press, E. G., & Nguyen, M. H. (2016). Aminoglycosides for treatment of bacteremia due to carbapenem-resistant *Klebsiella pneumoniae*. *Antimicrobial Agents and Chemotherapy*, 60(5), 3187–3192. <http://doi.org/10.1128/AAC.02638-15>
- Short, S. M., Chen, F., & Wilhelm, S. W. (2010). The construction and analysis of marker gene libraries. *Manual of Aquatic Viral Ecology*, 82–91. <http://doi.org/10.4319/mave.2010.978-0-9845591-0-7.82>

- Sridhar R. (2006). Bacteriophage. Retrieved from <http://microrao.com/micronotes/bacteriophage.pdf>.
- Subramaniam, G., Muniandy, S., & Parasakthi, N. (2005). SHV-5 extended-spectrum beta-lactamase from *Klebsiella pneumoniae* associated with a nosocomial outbreak in a paediatric oncology unit in Malaysia. *International Journal of Infectious Diseases*, 9(3), 170-172. doi:10.1016/j.ijid.2004.07.005
- Sulakvelidze, A., Alavidze, Z., & Morris, J. J. G. (2001). Minireview: Bacteriophage Therapy. *Antimicrobial Agents and Chemotherapy*, 45(3), 649-659. doi:10.1128/AAC.45.3.649-659.2001
- Sundar, M. M., Nagananda, G. S., Bhattachar, S., Suryan, S., & Das, A. (2009). Isolation of Host-Specific Bacteriophages from Sewage Against Human Pathogens. *Asian Journal of Biotechnology*, 1(4), 163-170. doi:10.3923/ajbkr.2009.163.170
- Suwantarat, N., & Carroll, K. C. (2016). Epidemiology and molecular characterization of multidrug-resistant Gram-negative bacteria in Southeast Asia. *Antimicrobial Resistance & Infection Control*, 5(1), 1-8. doi:10.1186/s13756-016-0115-6
- Szermmer-Olearnik, B., & Boratyński, J. (2015). Removal of Endotoxins from Bacteriophage Preparations by Extraction with Organic Solvents. *PloS One*, 10(3), 1-10. doi:10.1371/journal.pone.0122672
- Verma, P. (2007). Methods for Determining Bactericidal Activity and Antimicrobial Interactions: Synergy Testing, Time-Kill Curves, and Population Analysis. In R. Schwalbe, L. Steele-Moore, A. C. Goodwin (Eds.), *Antimicrobial Susceptibility Testing Protocols* (1st ed., pp. 275-298). Boca Raton, FL: CRC Press.

doi:<https://doi.org/10.1201/9781420014495>

- Viertel, T. M., Ritter, K., & Horz, H. (2014). Viruses versus bacteria--novel approaches to phage therapy as a tool against multidrug-resistant pathogens. *Journal of Antimicrobial Chemotherapy*, 69(9), 2326-2336. doi:10.1093/jac/dku173
- Weber-Dąbrowska, B., Jończyk-Matysiak, E., Żaczek, M., Łobocka, M., Łusiak-Szelachowska, M., & Górski, A. (2016). Bacteriophage Procurement for Therapeutic Purposes. *Frontiers in Microbiology*, 7, 1-14. doi:10.3389/fmicb.2016.01177
- Williams, S. H., Huggins, M. B., & Shaw, K. M. (1987). Factors Influencing the Survival and Multiplication of Bacteriophages in Calves and in Their Environment. *Journal of General Microbiology*, 133, 1127–1135. <http://doi.org/10.1099/00221287-133-5-1127>
- Wittebole, X., Roock, S. D., & Opal, S. M. (2014). A historical overview of bacteriophage therapy as an alternative to antibiotics for the treatment of bacterial pathogens. *Virulence*, 5(1), 226-235. doi:10.4161/viru.25991
- Wright, A., Hawkins, C. H., Anggard, E. E., & Harper, D. R. (2009). A controlled clinical trial of a therapeutic bacteriophage preparation in chronic otitis due to antibiotic-resistant *Pseudomonas aeruginosa*; a preliminary report of efficacy. *Clinical Otolaryngology*, 34(4), 349-357. doi:10.1111/j.1749-4486.2009.01973.x.
- Wu, L. T., Chang, S. Y., Yen, M. R., Yang, T. C., & Tseng, Y. H. (2007). Characterization of extended-host-range pseudo-T-even bacteriophage Kpp95 isolated on *Klebsiella pneumoniae*. *Applied and Environmental Microbiology*, 73(8), 2532–2540. <http://doi.org/10.1128/AEM.02113-06>
- Wu, S., Zachary, E., Wells, K., & Loc-Carrillo, C. (2013). Phage Therapy: Future Inquiries.

Postdoc Journal : A Journal of Postdoctoral Research and Postdoctoral Affairs, 1(6), 24–35.

- Wyres, K. L., & Holt, K. E. (2018). *Klebsiella pneumoniae* as a key trafficker of drug resistance genes from environmental to clinically important bacteria. *Current Opinion in Microbiology*, 45, 131–139. <http://doi.org/10.1016/j.mib.2018.04.004>
- Yong, D., Toleman, M. A., Giske, C. G., Cho, H. S., Sundman, K., Lee, K., & Walsh, T. R. (2009). Characterization of a new metallo- β -lactamase gene, blaNDM-1, and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. *Antimicrobial Agents and Chemotherapy*, 53(12), 5046–5054. <http://doi.org/10.1128/AAC.00774-09>
- You, L., Suthers, P. F., & Yin, J. (2002). Effects of *Escherichia coli* physiology on growth of phage T7 *in vivo* and *in silico*. *Journal of Bacteriology*, 184(7), 1888–1894. <https://doi.org/10.1128/jb.184.7.1888-1894.2002>
- Zajdowicz, S. L., & Holmes, R. K. (2016). Phage Conversion and the Role of Bacteriophage and Host Functions in Regulation of Diphtheria Toxin Production by *Corynebacterium diphtheriae*. In C. J. Hurst (Ed.), *The Mechanistic Benefits of Microbial Symbionts*, 2, 15-45. doi:10.1007/978-3-319-28068-4
- Zhao, F., Zhang, J., Fu, Y., Ruan, Z., & Xie, X. (2015). Dissemination of extensively drug-resistant and KPC-2 producing *Klebsiella pneumoniae* isolated from bloodstream infections. *The Journal of Infection in Developing Countries*, 9(9), 1016-1021. doi:10.3855/jidc.6679

APPENDICES

Appendix I

Ethical Approval

Fakulti Perubatan dan Sains Kesihatan
Faculty of Medicine and Health Sciences



UNIMAS/NC-21.02/03-02 Jld.2 (79)

3 Mei 2017

Dr Tan Cheng Siang
Fakulti Perubatan Dan Sains Kesihatan
Universiti Malaysia Sarawak
94300 Kota Samarahan,
Sarawak.

Dr,

Kelulusan Mesyuarat Etika Perubatan Untuk Kajian:
- *Bioprospecting of bacteriolytic bacteriophages infecting *Pseudomonas aeruginosa*.*

Sukacita dimaklumkan bahawa kertas kerja penyelidikan seperti di atas yang dijalankan oleh Dr Tan Cheng Siang dan penyelidik-penyelidik di bawah telah diluluskan dalam Mesyuarat Etika Perubatan Fakulti 03/2017 pada 2 Mei 2017.

Dr Siti Fairouz binti Ibrahim
Dr Samuel Lihan
Dr Azham bin Zulkharnain

Sehubungan itu, penyelidikan tersebut boleh dimulakan dan diharap ia dapat dijalankan seperti yang dirancang.

Sekian, terima kasih.

Yang benar,

Tan Sri Prof Datu Dr Mohamad Taha Arif
Pengerusi
Jawatankuasa Etika Perubatan
Fakulti Perubatan dan Sains Kesihatan

g/Chelham mesyuarat etika perubatan



● Universiti Malaysia Sarawak
94300 Kota Samarahan, Sarawak, MALAYSIA
● + 60 82 581 000
● + 60 82 665 152
● www.unimas.my

UNIMAS/NC-21.02/03-02 (79)

24 November 2015

Dr Tan Cheng Siang
Fakulti Perubatan dan Sains Kesihatan
Universiti Malaysia Sarawak
94300 Kota Samarahan

Dr,

Kelulusan Mesyuarat Etika Perubatan Untuk Kajian:

Prevalence of 3 Selected Microbes Among Medical Undergraduates In UNIMAS.

Dengan segala hormatnya perkara di atas dirujuk.

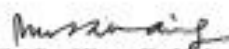
Sukacita dimaklumkan bahawa kertas kerja penyelidikan seperti di atas telah diluluskan dalam Mesyuarat Jawatankuasa Etika Perubatan Fakulti bil 04/2015 pada 23 November 2015. Projek tersebut dijalankan bersama pelajar-pelajar seperti nama di bawah yang diselin Dr Tan Cheng Siang.

- Ruth ak Dagang
- Mohd Amirul Husni bin Jakaria
- Wong May Yin
- Zuhairah Nasrin binti Haizan

Sehubungan itu, penyelidikan tersebut boleh dimulakan dan diharap ia dapat dijalankan seperti yang dirancang.

Sekian, terima kasih.

Yang benar,



Tan Sri Prof Datu Dr Mohamad Taha Arif
Pengerusi
Jawatankuasa Etika Perubatan
Fakulti Perubatan dan Sains Kesihatan

g/keputusan mesyuarat etika perubatan



Appendix II

Sampling Location



Domestic sewage sample collection from UNIMAS residential college,
College Kenanga.

Appendix III

16S rRNA Sequences of the Bacterial Samples

>1st_BASE_3005127_KP5 [Accession No.: MK386771]

GGGGCCGGGGGAGTGACCTACACATGCAAGTCGAGCGGTAGCACAGAGAGCT
TGCTCTCGGGTGACGAGCGGCGGACGGGTGAGTAATGTCTGGGAACTGCCTG
ATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAA
GACCAAAGTGGGGGACCTTCGGGCCTCATGCCATCAGATGTGCCCAGATGGGA
TTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCT
GAGAGGATGACCAGCCACACTGGAAGTGAAGACACGGTCCAGACTCCTACGGG
AGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGC
CGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAG
GCGATAAGGTTAATAACCTTGTGCGATTGACGTTACCCGCAGAAGAAGCACCGG
CTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGA
ATTACTGGGCGTAAAGCGCACGCGAGGCGGTCTGTCAAGTCGGATGTGAAATCC
CCGGGCTCAACCTGGGAAGTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAGA
GGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAAT
ACCGGTGGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAG
CGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGT
CGATTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAAT
CGACCGCCTGGGGAGTACGGCCGCAAGGTTAAACTCAAATGAATTGACGGG
GGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTTCGATGCAACGCCAAAAACC
TTACCTGGTCTTGACATCCACAGAACTTGCCAGAGATGGCTTGGTGCCCTTCGGG
AACTGTGAAACAGGTGCTGCATGGCTGTGCTCAGCTCGTGTGTGAAATGTTG
GGTTAAATCCCGCAACGAGCGCAACCCTTAACCTTTGTTTGCTGCGGTTTCGGTC
GGGGAAGTCAAAGAACTGCCATNATAAACTGGAGGAAAGGTGGGAATGACC
TCAAGTATCCTGGCCCTTACAACCAGGCCTGCCCGTGCTTAATTGGTATTAAAA
AAAAAACGACCCTCCAGGAACAAGCGGACCCTTAAAGTGGTCAATCCGGATTG
GATTNCACTTCAATCCCAGGAAGTAAACCGTTTAAACCGAAAACCAAATTTA
CCGTAAAAACTTTCCTGGCTTTTTTACCCCCCCTCCACCCTGGGGAATTGGTTT
AAAAAAATAAGGGCTTATCCCTTAGGGGGCTTAACTTTTGTAATTTTAGGGG
GTGAAATTAACCCATTGCCCATGGAAACACACGTGGGGTACCCCCCTAAAACC
TTTTTTTGGGGTTTATGGGTTTGTTTTTTGGGTTTTGGGTTTTGTTTTTTAAAAAT
ATTTTTTAATTTTTATAATTTTTATTTTAAAAAGGTTTTAATTTAAAAATTTTTT
AAAATTTTTTAAATAATTTTTTAAAAAATTTACCCCTTTTTTTTTTCCCCTTTTTT
AATTTTTTTTTTAAAAAACCCCTTTTTTTTTTACAACCTTTTTATTAAACCCTCAA
TCTTTTAATTCCCGCCCTTAATAATTTTCTATTATTTTACCGTTTTTACACCTTT
AAACACCTTTTTTTTTTTTAAACCCCTTTTTTTTAAAAAGAGAACCCCTTTATTAG
AATTAGAGGGGTGTTATAG

>1st_BASE_3005128_ESKP2

GCGCCATGGGTACGACTACACATGCAGTCGAGCGGTAGCACAGAGAGCTTGCT
CTCGGGTGACGAGCGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGG
AGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTGCAAGACC
AAAGTGGGGGACCTTCGGGCCTCATGCCATCAGATGTGCCCAGATGGGATTAG
CTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGA
GGATGACCAGCCACACTGGAAGTGAAGACACGGTCCAGACTCCTACGGGAGGC
AGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGT
GTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGGGGAGGAAGGCGAT
AAGGTTAATAACCTCGTCGATTGACGTTACCCGCAGAAGAAGCACCGGCTAAC
TCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTAC
TGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGG
CTCAACCTGGGAAGTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAGAGGGGG
GTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGT
GGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCAAGTGCGAAAGCGTGGG
GAGCAAACAGGATTAGATAACCCTGGTAGTCCACGCCGTAAACGATGTCGATTT
GGAGGTTGTGCCCTTGAGGCGTGGCTTCCTGAGCTAACGCGTTAAATCGACCG
CCTGGGGAGTACGGTCGCAAGGTTAAACTCATATGAATGGACTTGGCGCCCCG
TCACAACCGGTGGATCCTGTGGGTAAATTTCAATGCAACGCGGAGGAGCCTTT
CTTGGCCTTGAATTTCACTAAAACCTTCTCTACCTGAAATTGGTGCCCGTCGGC
TAATGGATAGAAAAGTAACTGGGTTGTTCTTCGGGCAGAATCATGTTTATCAA
TATGTAGGATTTAAGTCCCTACATCCCCTCTCAACCCCGTAAAAATTTTGTTC
CTTACTTTTACGGCCGTTCTCTTCCAAGGACATACTGGTTGGTTGTTTATTCTTG
TTAACTCTTTTGGTTAAATTGTTCTTCATTCCTTTTCCT

>1st_BASE_3005129_CRKP1 [Accession No.: MK386770]

GGCCGTTGCCTTCAGGCCTACACATGCAGTCGAGCGGTAGCACAGAGAGCTTG
CTCTCGGGTGACGAGCGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGAT
GGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTGCAAGA
CCAAAGTGGGGGACCTTCGGGCCTCATGCCATCAGATGTGCCCAGATGGGATT
AGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGA
GAGGATGACCAGCCACACTGGAAGTGAAGACACGGTCCAGACTCCTACGGGAG
GCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGC
GTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGGGGAGGAAGGCG
TTGAGGTTAATAACCTTGTCGATTGACGTTACCCGCAGAAGAAGCACCGGCTA
ACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATT
ACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCG
GGCTCAACCTGGGAAGTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAGAGGG
GGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACC
GGTGGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGT
GGGGAGCAAACAGGATTAGATAACCCTGGTAGTCCACGCCGTAAACGATGTCGA

TTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAATCGA
CCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGC
CCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTA
CCTGGTCTTGACATCCACAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAA
CTGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGT
TAAGTCCCGCAACCAGCGCAACCCTTATCCTTTGTTGCCAGCGGTTCCGGCCGGG
AACTCAAAGGAACTGCCAGTGATAAACTGGAAGAAAGGGGGGGGATGACGCC
AGTCCTCCTGGCCCTTACAACCAGGGCTACCACTGGGTAAATGGGCTATTCA
AAAAAATGAANCCCCAAAACAACCGGACCCCAAAAGTATTGCTAATCCGG
ATTGGATTTGCAATTCGCTTCCTGAAATCCGAATCCCTTAAAACCTAAAACCAA
ATGCCCCCGGTAAAATTTTCCGGGCCTTGAACCCCCCCTTCCACCTTGGAAG
GGTTTCCAAAAAATGAGGGGTTTAACCTTCGGAAGGGCTCCCCCTTGTAACT
TAACGGGGGGAATTTCAAAAAGTACCCTTGGGACCCCGGTGGTGTAACCTCCA
AATAAAGGCAAATTGCGAATTTAAATTAAGCATTTTTGGGGTTTTATTTTGGT
TTAATTTTAAGAATAAATTTTTTTATGGTTTTAATTTTTTAGGTTTTTTTAAATTT
TTTTTTTATATTTTGAATTTTTTTTATTTTAAAAACGTTTTTTTATATTTTTTTG
CCCCCGGGTTTTCAATTTAATGCCCCCCTTTATAAGCTATTTTTTATGTGGCGG
GTATTTATATAGCGTATTCACAAAAAAATTTTACGATTTAACCCCCCACTTTT
TTATTTTAAACCCCTTTATAACCCGCCCCCCCCCGCCCCCCCCCGCCCCCCCCC
CGGGGCCCCCCCCCCCC

>1st_BASE_3022445_KP6 [Accession No.: MK386786]

GGGCTTTGCGCAGTCTACACATGCAGTCGAGCGGTAGCACAGAGAGCTTGCTC
TCGGGTGACGAGCGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGG
AGGGGGATAACTACTGGAACGGTAGCTAATACCGCATAATGTCGCAAGACC
AAAGTGGGGGACCTTCGGGCCTCATGCCATCAGATGTGCCAGATGGGATTAG
CTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGA
GGATGACCAGCCACACTGGAAGTGAACACGGTCCAGACTCCTACGGGAGGC
AGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGT
GTGTGAAGAAGGCCTTCGGGTGTAAAGCACTTTCAGCGGGGAGGAAGGCGGT
GAGGTTAATAACCTTGTCGATTGACGTTACCCGCAGAAGAAGCACCGGCTAAC
TCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTAC
TGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGG
CTTAACCTGGGAAGTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAGAGGGGG
GTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGT
GGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGG
GAGCAAACAGGATTAGATAACCCTGGTAGTCCACGCCGTAAACGATGTCGATTT
GGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAATCGACCG
CCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGGCCCG
CACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCT
GGTCTTGACATCCACAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTG
TGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTA

AGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTCCGGCCGGGA
ACTCAAAGGAGACTGCCAGTGATAAACTGGAAGGAAGGTGGGGGATGACGTC
AAGTCATCATGGGCCCTTACGACCAGGGCTACCACCTGGCTACAATGGGCATT
AACAAAGAGAACCGACCTCCCGGAAGCAAGCGGACCTCATAAAGTATGTCCG
AATTCCGGATTGGGAGTCGCAACTCGACTCCCTGGAATCCGGAATCCCTTTTAA
TCCGGGAAATCAAAGGCTCCGGGGGAAAACTTTCCCGGGCCTTTTAAAACCC
GCCCCGTTCCCCCCCCGGGAGG

>1st_BASE_3022446_KP7 [Accession No.: MK386785]

CGCATGGGGGGGCAGGCTACACATGCAGTCGAGCGGTAGCACAGAGAGCTTG
CTCTCGGGTGACGAGCGGCGGACGGGTGAGTAATGTCTGGGAACTGCCTGAT
GGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGA
CCAAAGTGGGGGACCTTCGGGCCTCATGCCATCAGATGTGCCCAGATGGGATT
AGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGA
GAGGATGACCAGCCACACTGGAAGTGAAGACACGGTCCAGACTCCTACGGGAG
GCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGC
GTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGGGGAGGAAGGCG
GTGAGGTTAATAACCTTGTCGATTGACGTTACCCGCAGAAGAAGCACCGGCTA
ACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATT
ACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCG
GGCTCAACCTGGGAAGTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAGAGGG
GGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACC
GGTGGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGT
GGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGA
TTTGAGAGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAATCGA
CCGCCTGGGGAGTACGGCCGCAAGGTAAAACTCAAATGAATTGACGGGGGC
CCGCACAAGCGGTGGAGCATGTGGTTTAATTTCGATGCAACGCGAAGAACCTTA
CCTGGTCTTGACATCCACAGAAGTTTCCAGAGATGGATTGGTGCCTTCGGGAA
CTGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGT
TAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTCCGGCCGG
GAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTC
AAGTCATCATGGCCCTTACGACCAGGGCTACCACCGTGCTACAATGGCAATTA
CAAGAGAAACCGACCTCCCGAGAACAAGCGGACCTCAAAGTATGTCCTAATC
CGGATTGGAGTTGGCACCCCACTCCTGAAGTCGGAATCGCTTGAAATCGGAA
TACAAAAGCCCCGGGAAAACTTTCCGGGCCTTGTAACCGCCGTTAAA

>1st_BASE_3022448_KP8 [Accession No.: MK386783]

GCGGAGTTGGCGGCGGTCTACCATGCAGTCGAGCGGTAGCACAGAGAGCTTGC
TCTCGGGTGACGAGCGGCGGACGGGTGAGTAATGTCTGGGAACTGCCTGATG
GAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAATGTCGCAAGAC
CAAAGTGGGGGACCTTCGGGCCTCATGCCATCAGATGTGCCCAGATGGGATTA

GCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAG
AGGATGACCAGCCACACTGGAAGTGAAGACACGGTCCAGACTCCTACGGGAGG
CAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCG
TGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGGGGAGGAAGGCGA
TGAGGTAAATAACCTCATCGATTGACGTTACCCGCAGAAGAAGCACCGGCTAA
CTCCGTGCCAGCAGCCGCGGTAAATACGGAGGGTGCAAGCGTTAATCGGAATTA
CTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGG
GCTCAACCTGGGAAGTGCATTGAAACTGGCAGGCTAGAGTCTTGTAGAGGGG
GGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCG
GTGGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTG
GGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGAT
TTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAATCGAC
CGCCTGGGGAGTACGGCCGCAAGGTTAAACTCAAATGAATTGACGGGGGCC
CGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTAC
CTGGTCTTGACATCCACAGAACTTTCAGAGATGGATTGGTGCCTTCGGGAAGT
GTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTT
AAGTCCCCGAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTCCGGCCGGG
AACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCA
AGTCATCATGGCCCTTACGACCAGGGCTACCACGTGCTACAATGGCTTTACAA
AGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCAAAGTATGTCCGATTCCG
AATGAATTGGCACTCCACTCCCTGAAGTCGGAATCCCTGGAAACCGAAAACAA
AGGCCCCGGGAAAACCTTTCCCGGGCTTTT

>1st_BASE_3022449_KP9 [Accession No.: MK386782]

GGGATTGGCGGCAGTCTACCATGCAGTCGAGCGGTAGCACAGAGAGCTTGCTC
TCGGGTGACGAGCGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGG
AGGGGGATAACTACTGGAACGGTAGCTAATACCGCATAACGTCGCAAGACC
AAAGTGGGGGACCTTCGGGCCTCATGCCATCAGATGTGCCCAGATGGGATTAG
CTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGA
GGATGACCAGCCACACTGGAAGTGAAGACACGGTCCAGACTCCTACGGGAGGC
AGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGT
GTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGGGGAGGAAGGCGTT
AAGGTTAATAACCTTGTCGATTGACGTTACCCGCAGAAGAAGCACCGGCTAAC
TCCGTGCCAGCAGCCGCGGTAAATACGGAGGGTGCAAGCGTTAATCGGAATTAC
TGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGG
CTCAACCTGGGAAGTGCATTGAAACTGGCAGGCTAGAGTCTTGTAGAGGGGG
GTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGT
GGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGG
GAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGATTT
GGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAATCGACCG
CCTGGGGAGTACGGCCGCAAGGTTAAACTCAAATGAATTGACGGGGGGCCCG
CACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCT

GGTCTTGACATCCACAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTG
TGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTA
AGTCCCGCAACGAGCGCAACCCCTTATCCTTTGTTGCCAGCGGTTCCGGCCGGGA
ACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAAGGTGGGGGATGACGTC
AAGTCATCATGGCCCTTACGACCAGGGCTACACCCTTGCTACAATGGCCATTA
ACAAAGAGAAGCGACCTCGCGAGAACAAGCGGACCTCATAAAGTATGTCCTA
ATCCCGGATTGGAATCTGCCACTCAACCCCTGGAAAGGCCGGAATCCCTTGAA
ATCCGAAAATCAAAAAGCCTCCCGGGGAAAACTTTCCCGGGCCCTTTTAAGC
C

>1st_BASE_3022450_ESKP3 [Accession No.: MK386781]

CGATTTGCGGCAGCTACACATGCAGTCGAGCGGTAGCACAGAGAGCTTGCTCT
CGGGTGACGAGCGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGA
GGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAATGTCGCAAGACCA
AAGTGGGGGACCTTCGGGCCTCATGCCATCAGATGTGCCCAGATGGGATTAGC
TAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAG
GATGACCAGCCACACTGGAAGTGAAGACACGGTCCAGACTCCTACGGGAGGCA
GCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTG
TGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGGGGAGGAAGGCGTTG
AGGTTAATAACCTTGGCGATTGACGTTACCCGCAGAAGAAGCACCGGCTAACT
CCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACT
GGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGGC
TCAACCTGGGAACTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAGAGGGGGG
TAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTG
GCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGG
AGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGATTTG
GAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAATCGACCGC
CTGGGGAGTACGGCCGCAAGGTTAAACTCAAATGAATTGACGGGGGCCCCGC
ACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTG
GTCTTGACATCCACAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTGT
GAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTAA
GTCCCGCAACGAGCGCAACCCCTTTATCCTTTGTTGCCAGCGGTCCGGCCGGGA
ACTCAAAGGAGACTGCCAGTGATAAACTGGAAGGAAGGGGGGGATGACGTCA
AGTCATCATGGCCCTTACGACCAGGGTTACCACCTGGTTAAATGGCCTTTACAA
AAGAAACCGACCTCCCGGAAGCAAGCGGACCTCTAAAAGATTTCTAATCCGG
ATGGGAATTTGCAACCCGACCCTCCTGAAAGGGGAATCCCGGGAATTTGGGAA
TCAAAGG

>1st_BASE_3022451_KP10 [Accession No.: MK386784]

TGCTTGGCGACGGCCTACCATGCAAGTCGAGCGGTAGCACAGAGAGCTTGCTC
TCGGGTGACGAGCGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGG
AGGGGGATAACTACTGGAACGGTAGCTAATACCGCATAATGTGCGCAAGACC
GAAGTGGGGGACCTTCGGGCCTCATGCCATCAGATGTGCCCAGATGGGATTAG
CTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGA
GGATGACCAGCCACACTGGAAGTGAAGACACGGTCCAGACTCATAACGGGAGGC
AGCAGTGGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGT
GTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGGGGAGGAAGGCGAT
GAGGTTAATAACCTCATCGATTGACGTTACCCGCAGAAGAAGCACCGGCTAAC
TCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTAC
TGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGG
CTCAACCTGGGAACTGCATTTCGAAACTGGCAGGCTAGAGTCTTGTAGAGGGGG
GTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGT
GGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGG
GAGCAAACAGGATTAGATAACCCTGGTAGTCCACGCCGTAAACGATGTCGATTT
GGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAATCGACCG
CCTGGGGAGTACGGCCGCAAGGTTAAACTCAAATGAATTGACGGGGGCCCCG
CACAAGCGGTGGAGCATGTGGTTTAAATTCGATGCAACGCGAAGAACCTTACCT
GGTCTTGACATCCACAGAACTTTCAGAGATGGATTGGTGCCTTTCGGGAACT
GTGAAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGG
TTAAGTCCCCGCAACGAGCGCCACCCTTTATCCTTTGTTGCCAGCGGGTCCGGC
CGGGGAACTCAAAGGAAGACTGCCCGGTGATAAACTGGAAGGAAGGTTGGGG
ATGACCTTCAAGTCCATCATGGGCCCTTACCGAACCAGGGGCTACCCACGTGC
CTACAATGGGCTTTTTCAAAGGGAAACGACCTTCGCGAAAACCAAGCGGGACC
TTCATAAAGTTATGTTTTAAATTCC

>1st_BASE_3022452_ESKP4

TCGCAGTGGCGGCAGGCCTAACTCATGCAAGTCGAGCGCGTAGCACAGGAGA
GCTCTGCTCTCGGGTGACGAGCGGCGGACGGGTGAGTAATGTCTGGGAAACTG
CCTGATGGAGGGGGATAACTACTGGAACGGTTCGCTTATACCGCATAACGTGC
CAAGACCAAAGTGGGGGACCTTCGGGCCTCATGCCATCAGATGTGCCCAGATG
GGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGG
TCTGAGAGGATGACCAGCCACACTGGAAGTGAAGACACGGTCCAGACTCATAAC
GGAGGCAGCAGTGGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCAT
GCCGCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGGGGAGGA
AGGCGTTAAGGTTAATAACCTTGTGCGATTGACGTTACCCGCAGAAGAAGCACC
GGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCG
GAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAAT
CCCCGGGCTCAACCTGGGAACTGCATTTCGAAACTGGCAGGCTAGAGTCTTGT
GAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGA

ATACCGGTGGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAA
AGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGAT
GTCGATTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAA
ATCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGG
GGGCCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAAGA
ACCTTACCTGGGTCTTGACATCCACAGAACTTTTCCAGAGATGGATTGGGTGCC
CTTCGGGAACTGTGAAAACAGGTGGCTGCATGGCCTGTTTCGTCAGCCTCGTGTT
TGTGAAATGTTGGGGTTAAGTCCCGCCAACCGAGCGCCAACCCTTAATCCTTTT
GTTTGCCAGCGGGTTCGGCCCCGGGAACTCAAAGGAAGACTGGCCAGTGGATT
AAACTGGGAGGGAAGGGTGGGGGATGAACGTTCAAGTCCTTCATGGGCCCTT
TACGAACCAGGGCTTACACACCGGTGCTTACAAATGGGCAATATACA

>1st_BASE_3022453_KP11 [Accession No.: MK386779]

GCGCCTGGGCGGAGGCTACACATGCAAGTCGAGCGGTAGCACAGAGAGCTTG
CTCTCGGGTGACGAGCGGCGGACGGGTGAGTAATGTCTGGGAACTGCCTGAT
GGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAATGTCGCAAGA
CCAAAGTGGGGGACCTTCGGGCCTCATGCCATCAGATGTGCCCAGATGGGATT
AGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGA
GAGGATGACCAGCCACACTGGAAGTGGAGACACGGTCCAGACTCCTACGGGAG
GCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGC
GTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGGGGAGGAAGGCG
TTGAGGTTAATAACCTTGCGGATTGACGTTACCCGCAGAAGAAGCACCGGCTA
ACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATT
ACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCG
GGCTCAACCTGGGAACTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAGAGGG
GGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACC
GGTGGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGT
GGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGA
TTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAATCGA
CCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGC
CCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTA
CCTGGTCTTGACATCCACAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAA
CTGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGG
GTTAAGTCCCGCAACGAGCGCAACCCTTAATCCTTTGTTGCCAGCGGTCCGGCC
GGGGAAGTCAAAGGAGACTGCCAGTGATAAACTGGAAGGAAGGTGGGGGATG
ACGTCAAGTCATCATGGCCCCCTTACGACCAGGGGCTACCACCGTGGCTACAAT
GGCCATTAACAAAGAGAACCAACCTCCGCGAAAGCAAGCGGAAGTCTTAAAG
TATGTCCTAATCCGGAATGGGAATTTGGAAGTTCGACTCCCTGGAAGTCCGGA
ATCCCTAGAAATCGGAAAATCAAAAAGTTCACGGGGGAAATAATTTCCGGGGCC
TTTGAAACACCC

>1st_BASE_3022454_KP12 [Accession No.: MK386778]

GGGCAGGGCGGCAGGCTACACATGCAGTCGAGCGGTAGCACAGAGAGCTTGC
TCTCGGGTGACGAGCGGCGGACGGGTGAGTAATGTCTGGGAACTGCCTGATG
GAGGGGGATAACTACTGGAACGGTAGCTAATACCGCATAACGTCGCAAGAC
CAAAGTGGGGGACCTTCGGGCCTCATGCCATCAGATGTGCCCAGATGGGATTA
GCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAG
AGGATGACCAGCCACACTGGAAGTGAAGACACGGTCCAGACTCCTACGGGAGG
CAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCG
TGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGGGGAGGAAGGCGA
TAAGGTTAATAACCTTGTCGATTGACGTTACCCGCAGAAGAAGCACCGGCTAA
CTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTA
CTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGG
GCTCAACCTGGGAAGTGCATTTCGAAACTGGCAGGCTAGAGTCTTGTAGAGGGG
GGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCG
GTGGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTG
GGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGAT
TTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAATCGAC
CGCCTGGGGAGTACGGCCGCAAGGTTAAACTCAAATGAATTGACGGGGGCC
CGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTAC
CTGGTCTTGACATCCACAGAACTTTCAGAGATGGATTGGTGCCTTCGGGAAGT
GTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTT
AAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTCCGGCCGGG
AACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCA
AGTCATCATGGCCCTTACAACCAGGGCTACCACCGGGCTACAATGGCCATTAC
AAAGAGAAACCCACCTCCCGAAAGCAAGCGGACCTCATAAAGTATGTCCGAA
TCCCGGAATGGAATTTGGAAGTTGAATCCCTGGAATCGGAATCCCTTGAATAT
CCGAAACAAAAGGTCCCGGGAAAAACTTTCCCGGGCCTTTTAAACACCGCCCG
TCACCCTGGGGAAGGGGTTCCAAAAAAAAGGGGGGTTAACCTG

>1st_BASE_3022455_KP13

GCNGCATTGGCGGCGGTCTCAACATCTGAGTCGAGAGGTAGCGCGGGAGCTCG
GTCTCGGGTGTTGGGCTCGCTGACGGGGATGTCAAGGATGGAGACCTGCCTGAT
GACGATCTTCCTCCAGTGGGGATCGCCGCTTGTAGGGAATAGCGTCGGTTGAC
CGTAGTGGGGGACCTTCGGGCCTCAGGCCATCAGATGAGCCCAGATGGGATTA
CGTACTAGGTGGGGTAGCGGCCTCCCTGTGCTACGATCCCTATTTGGTCTGAGA
GGATGACCGGCCACATTGGAAGTGAAGACACGGTCCAGACTTATACGGGAGGC
AGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGT
GTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGGGGAGGAAGGCGAT
AAGGTTAATAACCTTGTCGATTGACGTTACCCGCAGAAGAAGCACCGGCTAAC
TCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTAC
TGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGG

CTCAACCTGGGAACTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAGAGGGGG
GTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGT
GGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGG
GAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGATTT
GGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAATCGACCG
CCTGGGGAGTACGGCCGCAAGGTAAACTCAAATGAATTGACGGGGGGCCCGC
ACAAGCGGTGGAGCATGTGGTTTAATTCGATGCACGCGAAAACCTTACCTGGT
CTTGACATCCAAGAACTTTCCGAGATGGATTGGTGCCTTCGGAACCTGGGAGAC
AGTGCTGCATGCTGTCGTCACCTCCGGTTGGGAATGTTGGGTAAAGTCCCCCACC
AGCCACCCCTTATCCTTGTTGCAGCGGTGCGCCGGGAACCAAAGGAACTGCCA
TGATAACTGGAGAAGGGGGGATAACCTCAGTCCTCTGCCCTTACAACAGGGCT
ACACCTGTTCAATGGCATTACAAAAAAAACCAACCCCGAAGAAAGGGACCTA
AAAAATTTCTAACCCGAATGGAATCGCACCCCCCCCCCGAAAGCGAAACCCGG
AAACCAAAAAAAAAGCCCGGGAGAATTTCCCGGCCTTTAACCCCCCCCCCCCC
GGGGGTGGTTGCAAAAAGGGGG

>1st_BASE_3022458_KA1 (*K. aerogenes*) [Accession No.: MK386775]

GCGCCTTGGCGGACGGTCTACACATGCAGTCGAGCGGTAGCACAGAGAGCTTG
CTCTCGGGTGACGAGCGGCGGACGGGTGAGTAATGTCTGGGAACTGCCTGAT
GGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTGCAAGA
CCAAAGTGGGGGACCTTCGGGCCTCATGCCATCAGATGTGCCCAGATGGGATT
AGCTAGTAGGTGGGGTAATGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGA
GAGGATGACCAGCCACACTGGAAGTGAAGACACGGTCCAGACTCCTACGGGAG
GCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGC
GTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGAGGAGGAAGGCG
TTAAGGTTAATAACCTTAGTGATTGACGTTACTCGCAGAAGAAGCACCGGCTA
ACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATT
ACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCG
GGCTCAACCTGGGAACTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAGAGGG
GGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACC
GGTGGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGT
GGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGA
CTTGAGAGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGA
CCGCCTGGGGAGTACGGCCGCAAGGTAAACTCAAATGAATTGACGGGGGC
CCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTA
CCTACTCTTGACATCCAGAGAACTTAGCAGAGATGCTTTGGTGCCTTCGGGAA
CTCTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGT
TAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTTAGGCCGG
GAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGGGGGGATGACGTC
AAGTCATCATGGCCCTTACAAGTAGGGCTACCACCGTGCTTAAATGGCCTTTAC
AAGAGAAAGCGACCTCCCGAAAGCAACCGGACCTCTAAAGTATGTCCGATTCC
GGATTGGAGTTCGCACCCGCACTCCTGAAGCCGGAATCCCTTGAAACCGAAAT

CAAAAGTTCCCGGGAAACTTTCCCGGGCCTTTTAACCCCCCCCCCCCCCTGG
GGG

>1st_BASE_3022457_KP15 [Accession No.: MK386773]

GTGAATTGGCGACGGTCTACACATGCAAGTCGAGCGGTAGCACAGAGAGCTTG
CTCTCGGGTGACGAGCGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGAT
GGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGA
CCAAAGTGGGGGACCTTCGGGCCTCATGCCATCAGATGTGCCCAGATGGGATT
AGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGA
GAGGATGACCAGCCACACTGGAAGTGAAGACACGGTCCAGACTCCTACGGGAG
GCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGC
GTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGGGGAGGAAGGCG
ATAAGGTTAATAACCTTGTCGATTGACGTTACCCGCAGAAGAAGCACCGGCTA
ACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATT
ACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCG
GGCTCAACCTGGGAACTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAGAGGG
GGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACC
GGTGGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGT
GGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGA
TTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAATCGA
CCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGC
CCGCACAAGCGGTGGAGCATGTGGTTTAATTGATGCAACGCGAAGAACCTTA
CCTGGTCTTGACATCCACAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAA
CTGTGAGACAGGTGCTGCATGGCTGTCTGCTCAGCTCGTGTGTGAAATGTTGGGT
TAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTCCGGCCGG
GAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTC
CAGTCATCATGGCCCTTACGACCAGGGCTACCACGTGCTACATGGCCATTACA
AGAGAAGCGACCTCGCAAGCCAGCGGACCTCATAAGTATGTCCGATTCCGGAT
TGGATTGCACTCGCCTCCTTAAGTCGGATCCCTTGAAATCGGAAACAAAAGG
TCCCCGGGAAACTTTCCGGGCCTTTAAACCCCCCCTCACCCCGGGGAAGGG
TTTCAAAAATAGGGGTTTAACCTTTGGAGGGCT

>1st_BASE_3005124_KA2 (*K. aerogenes*) [Accession No.: MK386774]

CGGCATGGGGGCAGCTACCATGCAGTCGAGCGGTAGCACAGAGAGCTTGCTCT
CGGGTGACGAGCGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGA
GGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCA
AAGTGGGGGACCTTCGGGCCTCATGCCATCAGATGTGCCCAGATGGGATTAGC
TAGTAGGTGGGGTAATGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAG
GATGACCAGCCACACTGGAAGTGAAGACACGGTCCAGACTCCTACGGGAGGCA
GCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTG
TATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGAGGAGGAAGGCGTTA

AGGTTAATAACCTTAGTGATTGACGTTACTCGCAGAAGAAGCACCGGCTAACT
 CCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACT
 GGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGGG
 TCAACCTGGGAACTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAGAGGGGGG
 TAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTG
 GCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGG
 AGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCTACTTG
 GAGGTTGTGTCCTTGAAGCGTGGCTTCCTGTAGCTAACGCGTTAAATTCTACCG
 CCTGGGGAGTACCGGCCGCTATGGTTAAAACTCAATTGAATTTGACTGGGGG
 CCCGCACAAGTCGGTGGAATCATGTGGGTTTACTTCCATGTCACCGTGAAAGA
 ACTTTATATTTACTTTTGATTTTCTTTAGTATTTAATCAAAATAGTTTATGGTGC
 TGTTTCGTAAACTTTAAATTATGGAGTTGGTTGGTTTGTGGGAATATTTTTGTTT
 TAGTAATGGTTGGATTACTTTTTTAACCCTAATGCCCATCCTGAATACTCTATT
 GTTAGCAGGTAAAGTTGCCTTTTCCTAATATTATTATTAGGTTTTGGTATTCTTT
 GTTATTAATTTTGTGGGATTTAATTAATTTGGTTGGGTTTTGTTTTTTTAAAT
 ATGGTTTTTTAATTAGGGATTTGAAATTTGAAGTATTTGTGGGGTTAATTTAAT
 AATTTGTTTAGGATTTGGGAATTTGGGGGTTATAAGGGGGTTAAAAATTTTTTT
 TAGAATTTTTAGATTTTTTGAATTTGGGGTTTTGGTTTTTAATTTTTTTAAGGAA
 TTTGAGTTTAATTTTGATTTTTAAGGTTTAATATAAGGGTTTTTAATTTTATTGG
 AAGTTTTTAAAAATTTTTTGCGGGTTAAAAGGTTTTACATTTCCGGGGGTTTAT
 TGCTTATTTTTTTAAAAGTTTAAAGATTTTTAAAGGGGGTAATTTTCGGAAAAATT
 ATTTTTGTAAAAAACCTTTCCCGGTTTTTTAACTCGTTGAGGGGGATTTTTTTA
 TTTTATGGGGCGCTTTTTACTTTGTAATATTGGGCGGTTTTTTTTTTATTAGCTTTT
 AGCCCCGTATTATGGGCCCCCTTTACAGGGTTGTGATATTTTAAAATTTTGTT
 ATTGGGGGTTTTATAGGGAAAAGTATATATAATTTTTTTTTAAGTTTTATTGGCC
 TTTTTTTATATTTTTTAAAACGGGTTTTTAATTTATTTGTTGGAAAATATTTATTTT
 TAATAAGGGGTTATAAGGATGTATAAAATTTTAGGGGGGGGTTTAAATTATAA
 AGGGGGGGGTTATATGTGTTTTTTAGGGGGGTTTTTTAAAAATTTTGTAGGGTT
 TAGAAAGTTTTTTGGGAGAATGGTTTTGGTTTTAGGGGGGTTTTTTGGGGTTTGG
 GGGGTTTTTTTGGGGGGGGTTTGGTGT

>1st_BASE_3011108_KP16 [Accession No.: MK386772]

TGGCCTGTCGGCAGCCTACCATGCAAGTCGAGCGGTAGCACAGAGAGCTTGCT
 CTCGGGTGACGAGCGGCGGACGGGTGAGTAATGTCTGGGAACTGCCTGATGG
 AGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAATGTCGCAAGACC
 AAAGTGGGGGACCTTCGGGCCTCATGCCATCAGATGTGCCCAGATGGGATTAG
 CTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGA
 GGATGACCAGCCACACTGGAAGTGAAGACACGGTCCAGACTCCTACGGGAGGC
 AGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGT
 GTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGGGGAGGAAGGCGAT
 AAGGTTAATAACCTTGTCGATTGACGTTACCCGCAGAAGAAGCACCGGCTAAC
 TCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTAC

TGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGG
CTCAACCTGGGAACTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAGAGGGGG
GTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGT
GGCGAAGGCGGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGG
GAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGATTT
GGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAATCGACCG
CCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGGCCCG
CACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCT
GGTCTTGACATCCACAGAACTTTCCAGAAATGGATTGGTGCCTTCGGGAACTG
TGAAACAGGTGCTGCATGGCTGTCGTCACCTCGTGTTGTGAAATGTTGGGTAA
GTCCCCACCAACCCAACCCTTATCCTTTGTTGCCACCGGTCCGGCCGGGAACT
CCAAGGAACTGCCATTTAAAACTGGAAGAAAGTGGGGGATAACTTCAAGTCT
CTGGCCCTTACAACCAGGGGCTACCCTTGCTACAAGGGCAATAACAAAGAAAAC
CACCTCCCCGAAGCAACCGGACCTCAAAAGTTTGCCCAATCCCGGATGGAATC
TGCAACCCGGATCCCTGAATCGAAACCGGGAATTCCGAAACCCAAAATTCCCA
GGAAATCTTTTCGGGGCCTTTAAACCCCCCCCCCTCCACCTGGGATTGGGTTC
AAAAAAAAAATGAAAACCAAACCTTCGGGAGGCCCTTACCTTTTGGTATTTCCA
TGGGGGGAAATCCAAAAAGAAACCTGGGAAACCGCCGTTGGGGCACCCCTAA
TAAAAAGGGTTAGGGGG

>1st_BASE_3011109_KP17 [Accession No.: MK386777]

GGCCAATGGCCGACAGACTACACATGCAAGTCGAGCGGTAGCACAGAGAGCT
TGCTCTCGGGTGACGAGCGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTG
ATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAA
GACCAAAGTGGGGGACCTTCGGGCCTCATGCCATCAGATGTGCCCAGATGGGA
TTAGCTGGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCT
GAGAGGATGACCAGCCACACTGGAAGTGAAGACACGGTCCAGACTCCTACGGG
AGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGC
CGCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGGGGAGGAAG
GCGGTGAGGTTAATAACCTCATCGATTGACGTTACCCGCAGAAGAAGCACCGG
CTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGA
ATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCC
CCGGGCTCAACCTGGGAACTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAGA
GGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAAT
ACCGGTGGCGAAGGCGGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAG
CGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAACGATGT
CGATTTGAGAGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAAT
CGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGG
GGCCCGCACAAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACC
TTACCTGGTCTTGACATCCACAGAACTTTCCAGAGATGGATTGGTGCCTTCGGG
AACTGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTG
GGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTTCCGGC

GGGAACTCAAAGGAACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACG
TCAAGTCATCATGGCCCTTACGACCAGGGCTACCAACTGCTACAATGGCATT
ACAAAAAAACCGACCTCCCCGAAACAAGCGGACCTCATAAAGTATTTCTTA
TCCGGATTGGATTTTCGCACTCCACTCCCTGAAATCCGGATCCCTTGAATCCGAA
ATCCAAATGCTCCGGGGAAAACGTTCCCGGGCCTTGGAAAACCGCCCCGTCCA
CCCTGGGGATGGGGTTGCCAAAAAAGGGGGGCTTAACCTTCCGGAAGGGCCT
TCCACTTTTGGTATTTAATGGGGGGGGAATCTAAAAGGATACCAGGAACAAGG
GCGCTTGGGGTAATCCTTAAAAATGAAAGGGTTG

>1st_BASE_3022456_KP14 [Accession No.: MK386776]

CGCATTGACTACGGTCTACACATGCAAGTCGAGCGGTAGCACAGAGAGCTTGC
TCTCGGGTGACGAGCGGCGGACGGGTGAGTAATGTCTGGGAACTGCCTGATG
GAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAATGTCGCAAGAC
CAAAGTGGGGGACCTTCGGGCCTCATGCCATCAGATGTGCCCAGATGGGATTA
GCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAG
AGGATGACCAGCCACACTGGAAGTGGAGACACGGTCCAGACTCCTACGGGAGG
CAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCG
TGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGGGGAGGAAGGCGG
TGAGGTTAATAACCTCATCGATTGACGTTACCCGCAGAAGAAGCACCGGCTAA
CTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTA
CTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGG
GCTCAACCTGGGAACTGCATTTCGAACTGGCAGGCTAGAGTCTTGTAGAGGGG
GGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCG
GTGGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTG
GGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGAT
TTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAATCGAC
CGCCTGGGGAGTACGGCCGCAAGGTTAAACTCAAATGAATTGACGGGGGCC
CGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTAC
CTGGTCTTGACATCCACAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACT
GTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTT
AAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTTAGGCCGGG
AACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCA
AGTCATCATGGCCCTTACAACCAGGGCTACCACCGTGCTACAATGGCTATTAC
AAAGGAAACCACCTCCCGAAACAAGCGGACCTCTAAAATATGTCCAATTCCGG
ATTGGATTGCACTCGATCCCTGAAATCGGAATCCCTTAAATCCGAAAACAAAA
GTCCCCCGGGAAAACCTTTTCCCCGGGCCTTTTAAACCCCCCGCTCCCCCGGGAA
GGGGTTCCAAAA

>1st_BASE_3011110_KO1 (*K. oxtoca*) [Accession No.: MK386769]

GGGCATGGCCGGCGGCTACACATGCAAGTCGACGGTAGCACAGAGAGCTTGCT
CTCGGGTGACGAGTGGCGGACGGGTGAGTAATGTCTGGGAACTGCCTGATGG
AGGGGGATAACTACTGGAACGGTAGCTAATACCGCATAACGTCGCAAGACC
AAAGAGGGGGACCTTCGGGCCTCTTGCCATCAGATGTGCCCAGATGGGATTAG
CTGGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGA
GGATGACCAGCCACACTGGAAGTGAAGACACGGTCCAGACTCCTACGGGAGGC
AGCAGTGGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGT
GTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCGAT
GAGGTTAATAACCTTGTCGATTGACGTTACCCGCAGAAGAAGCACCGGCTAAC
TCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTAC
TGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGG
CTCAACCTGGGAACTGCATTTCGAAACTGGCAGGCTGGAGTCTTGTAGAGGGGG
GTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGT
GGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGG
GAGCAAACAGGATTAGATAACCCTGGTAGTCCACGCTGTAAACGATGTCGACTT
GGAGGTTGTTCCCTTGAGGAGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCG
CCTGGGGAGTACGGCCGCAAGGTTAAACTCAAATGAATTGACGGGGGCCCCG
CACAAGCGGTGGAGCATGTGGTTTAATTTCGATGCAACGCGAAAAACCTTACCT
ACTCTTGACATCCACAGAACTTAGCAGAGATGCTTTGGTGCCTTCGGGAACTCT
GAAACAGGTGCTGCATGGCTGTCTGTCAGCTCGTGTTGTGAAATGTTGGGGTTA
AGTCCCGCCAACGAGCGCAACCCTTTATCCTTTGTTGCCAGCGGTCCGGCCCG
GAACTCAAAGGAAACTGCCAGTGATAAACTGGAAGGAAGGTGGGGGATGACG
TCAAGTCATCCTGGCCCCCTTACGAATAAGGGCTACCACCGTGGCTACAATGGG
CAATTACAAAGAAAAACCGACCTTCGGAAAACCAACCGGACCTTCAAAAAAT
ATTCCCTATTCCCGGATGGGAATTTGGCACCTCCACTCCCTGGAAATCGGAAAT
CCGTAGAAATCCGGGGATCCAAAATGCCCCCGGGGAAAAACTTTCCCGGGCCC
TTTTAAACCCCCCCCCCGTTAACCCATGGGGGAAGGGGGTTTCAAAAAAAAAA
TTGGGGTTTTTAAACTTTTTGGGAGGCCCTTCCAACCTTTTTGGTTTCTTAACCG
GGGGGGGAAGATTTTAAACAAGGACACCTGGGACCCCCTGTGCCGTTGCCTTTT
ACCCCTTCAACTATAAAAATAGGAGGT

Appendix IV

Housekeeping genes sequences of Bacterial Samples

1. ATCC KP

gapA:

GACGGTACCGTTGAAGTNAAAGACGGTCATCTGGTCGTTAACGGTAAAAAAT
CCGTGATACCGCTGAACGTGACCCGGCTAACCTGAAGTGGGACGAAGTTAGTG
TTGACGTTGTTGCTGAAGCAACCGGTATCTTCCTGACCGACGAAACCGCTCGTA
AACACATCACCGCTGGCGCGAAAAAAGTCGTTCTGACTGGCCCGTCCAAAGAC
AACACTCCGATGTTTCGTTTCGCGGCGCTAACTTCGACGCTTACGCTGGCCAGGA
CATCGTTTCCAACGCTTCCTGCACCACCAACTGCCTGGCGCCGCTGGCTAAAGT
TATCAACGACAACCTTCGGTATCGTTGAAGGCCTGATGACCACCGTCCACGCTA
CCACCGCTACTCAGAAAACCGTTGATGGCCCGTCTCACAAGACTGGCGCGGC
GGCCGCGGCGCAGCTCAGAACATCATCCCGTCCTCTACCGGCGCTGCTAAAGC
AGTAGGTAAAGTACTGCCAGAACTGAACGGCAAACCTGACCGGTATGGCGTTCC
GCGTTCCAACCTCCGAACGTATCTGTTGTTGACCTGACCGTTCGTCTGGAAAAAG
CAGCGTCCTACGAAGAAATCAAGAAAGCCATCAAAGCCGCTTCTGAAGGAAA
TTGTTATCCCCCTTCACAAAA

rpoB:

GCGTTGGCCTGGTACGTGTAGAGCGTGCGGTGAAAGAGCGTCTGTCTCTTGGC
GATCTGGATACCCTGATGCCTCAGGATATGATCAACGCCAAGCCGATTTCCGC
AGCAGTGAAAGAGTTCTTTGGTTCCAGCCAGCTGTCTCAGTTTATGGACCAGA
ACAACCCGCTGTCTGAGATTACGCACAAACGTCGTATCTCCGCACTCGGCCCA
GGCGGTCTGACCCGTGAGCGCGCAGGCTTCGAAGTTCGAGACGTACACCCGAC
CCACTACGGTCGCGTATGTCCGATCGAAACGCCTGAAGGTCCGAACATCGGTC
TGATTAACCTCCCTGTCCGTGTACGCGCAGACCAACGAATATGGCTTCCTTGAGA
CGCCGTATCGTAAAGTGACCGACGGTGTGGTTACTGACGAAATTCACTACCTG
TCTGCTATCGAAGAAGGCAACTACGTTATCGCTCAGGCGAACTCCAACCTGGA
TGAAAACGGCCACTTCGTAGAAGATCTGGTTACCTGCCGTAGCAAAGGCGAAT
CCAGCTTGTTTCAGCCGCGACCAGGTTGACTACATGGACGTATCCACCCAGCAG
GTGGTATCCGTTCGGTTCGCTCCCTGATCCCGTTCCTGGAACACGATGACGCCAA
CCGTGCATTGATGGGTGCGAACATGCAACGTCAGGCGGTTCCGACTCTGCGCG
CTGATAAGCCGCTGGTTGGTACCGGTATGGAACGTGCTGTTGCCGTTGACTCCG
GTGTTACTGCCGTGGCTAAACGTGGCGGTACCGTTCAGTACGTGGATGCTTCCC
GTATCGTTATCAAAGTTAACGAAGACGAGATGTACCCGGGCGAAGCAGGTATC
GACATCTATAACCTGACCAAGTACACCCGTTCTAACCAGAACACCTGCATCAA
CCAGATGCCTTGCGTGTCCCTGGGCGAACCTATTGAGCGCGGCGACGTGCTGG
CAGACGGCCCGTCCACCGACCTCGGTGAGCTGGCGCTGGGTGAGAACATGCGT

GTAGCGTTCATGCCGTGGAACGGTTACACTTCGAGACTCGAATNNTTTNNCNC
NNTCAAAAAAAAAA

tonB:

GAACAGCCTTCTCCGACGCAGCCGATAGAGATCACAATGGTGGCGCCGGCCGA
TCTTGAGCCGCCTCCGGCGGCGCAGCCTGTCGTGGAGCCCGTTGTTGAACCCG
AACCTGAGCCGGAGCCAGAGGTAGCGCCTGAACCGCCGAAAGAGGCGCCGGT
GGTGATCCATAAACCGGAACCTAAGCCGAAGCCCAAACCTAAACCCAAGCCTA
AGCCGGAGAAAAAGGTTGAACAGCCGAAGCGGGAAGTGAAGCCGGCAGCAG
AGCCGCGTCCGGCCTCGCCGTTTGAACAACAATACGGCGCCGGCGCGGTACA
GCGCCAAGTACCTCGACCGCAGCGGCTAAACCCACCGTTACTGCTCCGAGCGG
CCCGCGGGCGATCAGCCGCGTTCAGCCGTCCTATCCGGCGCGCGCTCAGGCGC
TGCGCATTGAAGGGACGGTACGGGTGAAGTTTGACGTTTCGCCTGATGGCCGC
ATTGATAATCTGCAGATCCTCTCTGCCAGCCGGCGAATGAAATTGTTATCGGC
CTCACAAAA

2. CRKP1

gapA:

CGGACGGTACCGTTGAAGTGAAAGACGGTCATCTGGTCGTTAACGGTAAAAAA
ATCCGTGTTACCGCTGAACGTGACCCGGCTAACCTGAAGTGGGACGAAGTTAG
TGTTGACGTTGTTGCTGAAGCAACCGGTATCTTCCTGACCGACGAAACCGCTCG
TAAACACATCACCGCTGGCGCGAAAAAAGTCGTTCTGACTGGCCCGTCCAAAG
ACAACACTCCGATGTTCTGTTTCGCGGCGCTAACTTCGACGCTTACGCAGGCCAG
GACATCGTTTCCAACGCTTCCTGCACCACCAACTGCCTGGCGCCGCTGGCTAAA
GTTATCAACGACAACTTCGGTATCGTTGAAGGCCTGATGACCACCGTCCACGC
TACCACCGCTACTCAGAAAACCGTTGATGGCCCGTCTCACAAAGACTGGCGCG
GCGGCCGCGGCGCAGCTCAGAACATCATCCCGTCTCTACCGGCGCTGCTAAA
GCAGTAGGTAAAGTACTGCCAGAACTGAACGGCAAACCTGACCGGTATGGCGTT
CCGCGTTCCGACTCCGAACGTATCTGTTGTTGACCTGACCGTTCGTCTGGAAAA
AGCAGCGTCCTACGAAGAAATCAAGAAAGCCATCAAAGCCGCTTCTGAAGGA
AATTGTTATCCGCCCCCTTCACAAAAA

rpoB:

GCGTTGGCCTGGTACGTGTAGAGCGTGCGGTGAAAGAGCGTCTGTCTCTTGGC
GATCTGGATACCCTGATGCCTCAGGATATGATCAACGCCAAGCCGATTTCCGC
AGCAGTGAAAGAGTTCTTTGGTTCCAGTCAGCTGTCTCAGTTTATGGACCAGA
ACAACCCGCTGTCTGAGATTACGCACAAACGTCGTATCTCCGCACTCGGCCCA
GGCGGTCTGACCCGTGAGCGCGCAGGCTTCGAAGTTCGAGACGTACACCCGAC
CCACTACGGTCGCGTATGTCCGATCGAAACGCCTGAAGGTCCGAACATCGGTC
TGATTAACCTCCCTGTCCGTGTACGCGCAGACCAACGAATATGGCTTCCTTGAGA
CGCCGTATCGTAAAGTGACCAACGGTGTGGTTACTGACGAAATTCACTACCTG

TCTGCTATCGAAGAAGGCAACTACGTTATCGCTCAGGCGAACTCCAACCTGGA
TGAAAACGGCCACTTCGTAGAAGATCTGGTTACCTGCCGTAGCAAAGGCGAAT
CCAGCTTGTTTCAGCCGCGACCAGGTTGACTACATGGACGTATCCACCCAGCAG
GTGGTATCCGTCGGTGCCTCCCTGATCCCGTTCCCTGGAACACGATGACGCCAA
CCGTGCATTGATGGGTGCGAACATGCAACGTCAGGCGGTTCCGACTCTGCGCG
CTGATAAGCCGCTGGTTGGTACCGGTATGGAACGTGCTGTTGCCGTTGACTCCG
GTGTTACTGCCGTGGCTAAACGTGGCGGTACCGTTCAGTACGTGGATGCTTCCC
GTATCGTTATCAAAGTTAACGAAGACGAGATGTACCCGGGCGAAGCAGGTATC
GACATCTATAACCTGACCAAGTACACCCGTTCTAACCCAGAACACCTGCATCAA
CCAGATGCCTTGCGTGTCCCTGGGCGAACCTATTGAGCGCGGCGACGTGCTGG
CAGACGGCCCGTCCACCGACCTCGGTGAGCTGGCGCTGGGTCAGAACATGCGT
GTAGCGTTCATGCCGTGGAACGGTTACACTTCNAGATCGAATGATTNCCCNCC
CCCCAAAAAAA

tonB:

ACAGCCTTCTCCGACGCAGCCGATAGAGATCACAATGGTGGCGCCGGCCGATC
TTGAGCCGCCTCCGGCGGCGCAGCCTGTCTGTGGAGCCCGTTGTTGAACCCGAA
CCTGAGCCGGAGCCAGAGGTAGTGCCTGAACCGCCGAAAGAGGCGCCGGTGG
TGATCCATAAACCGGAACCTAAGCCGAAGCCCAAACCTAAACCCAAGCCTAAG
CCGGAGAAAAAGGTTGAACAGCCGAAGCGGGAAGTGAAGCCGGCAGCAGAGC
CGCGTCCGGCCTCGCCGTTTGAAAACAACAATACGGCGCCGGCGCGTACAGCG
CCAAGCACCTCGACAGCAGCGGCTAAACCCACCGTTACTGCTCCAAGCGGCCC
GCGGGCGATCAGCCGCGTTCAGCCGTCCTATCCAGCGCGCGCTCAGGCGCTGC
GCATTGAAGGTACGGTACGGGTGAAGTTTGACGTTTCGCCTGATGGCCGTATT
GATAATCTGCAGATCCTCTCTGCTCAGCCGGCGAATGAAATTGTTATCCGGCTC
ACAAAA

3. ESKP2

gapA:

NNNNNNNNNTCNGACGGTACCGTTGAAGTGAAAGACGGTCATCTGGTCGTTAA
CGGTAAAAAATCCGTGATACCGCTGAACGTGACCCGGCTAACCTGAAGTGGG
ACGAAGTTAGTGTTGACGTTGTTGCTGAAGCAACCGGTATCTTCCTGACCGAC
GAAACCGCTCGTAAACACATCACCGCTGGCGCGAAAAAAGTCGTTCTGACTGG
CCCGTCCAAAGACAACACTCCGATGTTTCGTTTCGCGGCGCTAACTTCGACGCTTA
CGCTGGCCAGGACATCGTTTCCAACGCTTCCTGCACCACTAACTGCCTGGCGCC
GCTGGCTAAAGTTATCAACGACAACCTTCGGTATCGTTGAAGGCCTGATGACCA
CCGTCCACGCTACCACCGCTACTCAGAAAACCGTTGATGGCCCGTCTCACAAA
GACTGGCGCGGCGGCGGCGCAGCTCAGAACATCATCCCGTCTCTACCGG
CGCTGCTAAAGCAGTAGGTAAAGTACTGCCAGAACTGAACGGCAAACCTGACC
GGTATGGCGTTCCGCGTTCCGACTCCGAACGTATCTGTTGTTGACCTGACCGTT

CGTCTGGAAAAAGCTGCGTCCTACGAAGAAATCAAGAAAGCCATCAAAGCCG
CTTCTGAAGGAAATTGTTATCCGGCCTTCACAAAAAA

rpoB:

CGTTGGCCTGGTACGTGTAGAGCGTGCGGTGAAAGAGCGTCTGTCTCTTGGCG
ATCTGGATACCCTGATGCCTCAGGATATGATCAACGCCAAGCCGATTTCCGCA
GCAGTGAAAGAGTTCTTTGGTTCCAGCCAGCTGTCTCAGTTTATGGACCAGAA
CAACCCGCTGTCTGAGATTACGCACAAACGTCGTATCTCCGCACTCGGCCAG
GCGGTCTGACCCGTGAGCGCGCAGGCTTCGAAGTTCGAGACGTACACCCGACC
CACTACGGTCGCGTATGTCCGATCGAAACGCCTGAAGGTCCGAACATCGGTCT
GATTAACCTCCCTGTCCGTGTACGCGCAGACCAACGAATATGGCTTCCTTGAGA
CGCCGTATCGTAAAGTGACCGACGGTGTGGTTACTGACGAAATTCACTACCTG
TCTGCTATCGAAGAAGGCAACTACGTTATCGCTCAGGCGAACTCCAACCTGGA
TGAAAACGGCCACTTCGTAGAAGATCTGGTTACCTGCCGTAGCAAAGGCGAAT
CCAGCTTGTTTCAGCCGCGACCAGGTTGACTACATGGACGTATCCACCCAGCAG
GTGGTATCCGTCGGTGCGTCCCTGATCCCGTTCCTGGAACACGATGACGCCAA
CCGTGCATTGATGGGTGCGAACATGCAACGTCAGGCGGTTCCGACTCTGCGCG
CTGATAAGCCGCTGGTTGGTACCGGTATGGAACGTGCTGTTGCCGTTGACTCCG
GTGTTACTGCCGTGGCTAAACGTGGCGGTACCGTTCAGTACGTGGATGCTTCCC
GTATCGTTATCAAAGTTAACGAAGACGAGATGTACCCGGGCGAAGCAGGTATC
GACATCTATAACCTGACCAAGTACACCCGTTCTAACCAGAACACCTGCATCAA
CCAGATGCCTTGCGTGTCCCTGGGCGAACCTATTGAGCGCGGCGACGTGCTGG
CAGACGGCCCGTCCACCGACCTCGGTGAGCTGGCGCTGGGTCAGAACATGCGT
GTAGCGTTCATGCCGTGGAACGGTTACAACCTTCGAGATCGAATGATTTCCCCC
NCCAAAAAAA

tonB:

AACAGCCTTCTCCGACGCAGCCGATAGAGATCACAATGGTGGCGCCGGCCGAT
CTTGAGCCGCCTCCGGCGGGCGCAGCCTGTCTGAGAGCCCGTTGTTGAACCCGA
ACCTGAGCCGGAGCCAGAGGTAGCGCCTGAACCGCCGAAAGAGGCGCCGGTG
GTGATCCATAAACCGGAACCTAAGCCGAAGCCCAAACCTAAACCCAAGCCTAA
GCCGGAGAAAAAGGTTGAACAGCCGAAGCGGGAAGTGAAGCCGGCAGCAGA
GCCGCGTCCGGCCTCGCCGTTTGAAAACAACAATACGGCGCCGGCGCGTACAG
CGCCAAGTACCTCGACCGCAGCGGCTAAACCCACCGTTACTGCTCCGAGCGGC
CCGCGGGCGATCAGCCGCGTTCAGCCGTCTATCCGCCGCGCGCTCAGGCGCT
GCGCATTGAAGGGACGGTACGGGTGAAGTTTGACGTTTCGCCTGATGGCCGCA
TTGATAATCTGCAGATCCTCTCTGCTCAGCCGGCGAATGAAATTGTTATCGGCC
TCACAAAA

4. KP6

gapA:

GTTTCGACGGTACCGTTGAAGTGAAAGACGGTCATCTGGTCGTTAACGGTAAA
AAAATCCGTGTTACCGCTGAACGTGACCCGGCTAACCTGAAGTGGGACGAAGT
TGGTGTGACGTTGTTGCTGAAGCAACCGGTATCTTCCTGACCGACGAAACCG
CTCGTAAACACATCACCGCTGGCGCGAAAAAAGTCGTTCTGACTGGCCCGTCC
AAAGACAACACTCCGATGTTTCGTTTCGCGGCGCTAACTTCGACGCTTACGCTGG
CCAGGACATCGTTTCCAACGCTTCCTGCACCACCAACTGCCTGGCGCCCGTGGC
TAAAGTTATCAACGACAACCTTCGGTATCGTTGAAGGCCTGATGACCACCGTCC
ACGCTACCACCGCTACTCAGAAAACCGTTGATGGCCCGTCTCACAAAGACTGG
CGCGGCGGCCGCGGCGCAGCTCAGAACATCATCCCGTCCTCTACCGGCGCTGC
TAAAGCAGTAGGTAAAGTACTGCCAGAACTGAACGGCAAACCTGACCGGTATG
GCGTTCCGCGTTCCGACTCCGAACGTATCTGTTGTTGACCTGACCGTTCGTCTG
GAAAAAGCAGCGTCCTACGAAGAAATCAAGAAAGCCATCAAAGCCGCTTCTG
AAGGAAATTGTTATCCCGCCTTCACAAAA

rpoB:

CGTTGGCCTGGTACGTGTAGAGCGTGCGGTGAAAGAGCGTCTGTCTCTTGGCG
ATCTGGATACCTGATGCCTCAGGATATGATCAACGCCAAGCCGATTTCCGCA
GCAGTGAAAGAGTTCTTTGGTTCTAGCCAGCTGTCTCAGTTTATGGACCAGAAC
AACCCGCTGTCTGAGATTACGCACAAACGTCGTATCTCCGCACTCGGCCAGG
CGGTCTGACCCGTGAGCGCGCAGGCTTCGAAGTTCGAGACGTACACCCGACCC
ACTACGGTCGCGTATGTCCGATCGAAACGCCTGAAGGTCCGAACATCGGTCTG
ATTAACCTCCCTGTCCGTGTACGCGCAGACCAACGAATATGGCTTCCTTGAGAC
GCCGTATCGTAAAGTGACCGACGGTGTGGTACTGACGAAATTCACTACCTGT
CTGCTATCGAAGAAGGCAACTACGTTATCGCTCAGGCGAACTCCAACCTGGAT
GAAAACGGCCACTTCGTAGAAGATCTGGTTACCTGCCGTAGCAAAGGCGAATC
CAGCTTGTTTCAGCCGCGACCAGGTTGACTACATGGACGTATCCACCCAGCAGG
TGGTATCCGTCCGTGCGTCCCTGATCCCGTTCCTGGAACACGATGACGCCAACC
GTGCATTGATGGGTGCGAACATGCAACGTCAGGCGGTTCGACTCTGCGCGCT
GATAAGCCGCTGGTTGGTACCGGTATGGAACGTGCTGTTGCCGTTGACTCCGG
TGTTACTGCCGTGGCTAAACGTGGCGGTACCGTTCAGTACGTGGATGCTTCCCCG
TATCGTTATCAAAGTTAACGAAGACGAGATGTACCCGGGCGAAGCAGGTATCG
ACATCTATAACCTGACCAAGTACACCCGTTCTAACCAGAACACCTGCATCAAC
CAGATGCCTTGCGTGTCCCTGGGCGAACCTATTGAGCGCGGCGACGTGCTGGC
AGACGGCCCGTCCACCGACCTCGGTGAGCTGGCGCTGGGTCAGAACATGCGTG
TAGCGTTCATGCCGTGGAACGGTTACAACCTTCGAGACTCGAATGTTTTCCCCC
CTCAAAAAAAG

tonB:

CCTTCTCCGACGCAGCCGATAGAGATCACAATGGTGGCGCCGGCCGATCTTGA
GCCGCTCCGGCGGCGCAGCCTGTCGTGGAGCCCGTTGTTGAACCCGAACCTG
AGCCGGAGCCAGAGGTAGTGCCTGAACCGCCGAAAGAGGGCGCCGGTGGTGAT
CCATAAACCGGAACCTAAGCCGAAGCCCAAACCTAAACCCAAGCCTAAGCCG
GAGAAAAAGGTTGAACAGCCGAAGCGGGAAGTGAAGCCGGCTGCAGAGCCGC
GTCCGGCCTCGCCGTTTGAAAACAACAATACGGCGCCGGCGCGTACAGCGCCA
AGCACCTCGACAGCAGCGGCTAAACCCACCGTTACTGCTCCAAGCGGCCCGCG
GGCGATCAGCCGCGTTCAGCCGTCTATCCGGCGCGCGCTCAGGCGCTGCGCA
TAGAAGGTACGGTACGGGTGAAGTTTGACGTTTCGCCTGATGGCCGCATTGAT
AATCTGCAGATCCTCTCTGCTCAGCCGGCGAATGAAATTGTTATCGGCCTCACA
AAA

5. KP7

gapA:

TTTCGACGGTACCGTTGAAGTGAAAGACGGTCATCTGGTCGTTAACGGTAAAA
AAATCCGTGTTACCGCTGAACGTGACCCGGCTAACCTGAAGTGGGACGAAGTT
GGTGTGACGTTGTTGCTGAAGCAACCGGTATCTTCCTGACCGACGAAACCGC
TCGTAAACACATCACCGCTGGCGCGAAAAAAGTCGTTCTGACTGGCCCGTCCA
AAGACAACACTCCGATGTTTCGTTTCGCGGCGCTAACTTCGACGCTTACGCTGGC
CAGGACATCGTTTCCAACGCTTCCTGCACCACCAACTGCCTGGCGCCGCTGGCT
AAAGTTATCAACGACAACCTTCGGTATCGTTGAAGGCCTGATGACCACCGTCCA
CGCTACCACCGCTACTCAGAAAACCGTTGATGGCCCGTCTCACAAAGACTGGC
GCGGCGGCCGCGGCGCAGCTCAGAACATCATCCCGTCTCTACCGGCGCTGCT
AAAGCAGTAGGTAAAGTACTGCCAGAAGTGAACGGCAAAGTACCGGTATGG
CGTTCCGCGTTCGACTCCGAACGTATCTGTTGTTGACCTGACCGTTCGTCTGG
AAAAAGCAGCGTCCTACGAAGAAATCAAGAAAGCCATCAAAGCCGCTTCTGA
AGGAAATTGTTATCCGCCCTTCACAAAAA

rpoB:

GCGTTGGCCTGGTACGTGTAGAGCGTGCGGTGAAAGAGCGTCTGTCTCTTGGC
GATCTGGATACCCTGATGCCTCAGGATATGATCAACGCCAAGCCGATTTCCGC
AGCAGTGAAAGAGTTCTTTGGTTCCAGCCAGCTGTCTCAGTTTATGGACCAGA
ACAACCCGCTGTCTGAGATTACGCACAAACGTCGTATCTCCGCACTCGGCCCA
GGCGGTCTGACCCGTGAGCGCGCAGGCTTCGAAGTTCGAGACGTACACCCGAC
CCACTACGGTCGCGTATGTCCGATCGAAACGCCTGAAGGTCCGAACATCGGTC
TGATTAACCTCCCTGTCCGTGTACGCGCAGACCAACGAATATGGCTTCCTTGAGA
CGCCGTATCGTAAAGTGACCGACGGTGTGGTTACTGACGAAATTCCTACCTG
TCTGCTATCGAAGAAGGCAACTACGTTATCGCTCAGGCGAACTCCAACCTGGA
TGAAAACGGCCACTTCGTAGAAGATCTGGTTACCTGCCGTAGCAAAGGCGAAT
CCAGCTTGTTTCAGCCGCGACCAGGTTGACTACATGGACGTATCCACCCAGCAG

GTGGTATCCGTCGGTGCGTCCCTGATCCCGTTCCTGGAACACGATGACGCCAA
CCGTGCATTGATGGGTGCGAACATGCAACGTCAGGCGGTTCCGACTCTGCGCG
CTGATAAGCCGCTGGTTGGTACCGGTATGGAACGTGCTGTTGCCGTTGACTCCC
GTGTTACTGCCGTGGCTAAACGTGGCGGTACCGTTCAGTACGTGGATGCTTCCC
GTATCGTTATCAAAGTTAACGAAGACGAGATGTACCCGGGCGAAGCAGGTATC
GACATCTATAACCTGACCAAGTACACCCGTTCTAACCAGAACACCTGCATCAA
CCAGATGCCTTGCGTGTCCCTGGGCGAACCTATTGAGCGCGGCGACGTGCTGG
CAGACGGCCCGTCCACCGACCTCGGTGAGCTGGCGCTGGGTCAGAACATGCGT
GTAGCGTTCATGCCGTGGAACGGTTACAACCTTCGAGACNAANGATTTCTNNCN
TCAAAAAAAAAAAG

tonB:

AACAGCCTTCTCCGACGCNCCCGATGAGAGATNANANTGNCGGCTCCCNAACG
ATCTTGAGCCGCCTCCGGCGGCGCAGCCTGTGCTGGAGCCCGTTGTTGAACCC
GAACCTGAGCCGGAGCCAGAGGTAGTGCCTGAACCGCCGAAAGAGGGCGCCGG
TGGTGATCCATAAACCGGAACCTAAGCCGAAGCCCAAACCTAAACCCAAGCCT
AAGCCGGAGAAAAAGGTTGAACAGCCGAAGCGGGAAGTGAAGCCGGCTGCGG
AGCCGCGTCCGGCCTCGCCGTTTGAAAACAACAATACGGCGCCGGCGCGTACA
GCGCCAAGTACCTCGACCGCAGCGGCTAAACCCACCGTTACTGCTCCGAGCGG
CCCGCGGGCGATCAGCCGCGTTCAGCCGTCTATCCGGCGCGCGCTCAGGCGC
TGCGCATTGAAGGGACGGTACGGGTGAAGTTTGACGTTTCGCCTGATGGCCGC
ATTGATAATCTGCAGATCCTCTCCGCCAGCCGGCGAATGAAATTGTTATCGGC
CTCACAAAA

6. KP8

gapA:

GTTTCGACGGTACCGTTGAAGTGAAAGACGGTCATCTGGTCGTTAACGGTAAA
AAAATCCGTGTTACCGCTGAACGTGACCCGGCTAACCTGAAGTGGGACGAAGT
TGGTGTTGACGTTGTTGCTGAAGCAACCGGTATCTTCCTGACCGACGAAACCG
CTCGTAAACACATCACCGCTGGCGCGAAAAAAGTCGTTCTGACTGGCCCGTCC
AAAGACAACACTCCGATGTTTCGTTTCGCGGCGCTAACTTCGACGCTTACGCTGG
CCAGGACATCGTTTCCAACGCTTCCTGCACCACCAACTGCCTGGCGCCGCTGGC
TAAAGTTATCAACGACAACCTTCGGTATCGTTGAAGGCCTGATGACCACCGTCC
ACGCTACCACCGCTACTCAGAAAACCGTTGATGGCCCGTCTCACAAAGACTGG
CGCGGCGGCCGCGGCGCAGCTCAGAACATCATCCCGTCCTCTACCGGCGCTGC
TAAAGCAGTAGGTAAAGTACTGCCAGAACTGAACGGCAAACCTGACCGGTATG
GCGTTCCGCGTTCCGACTCCGAACGTATCTGTTGTTGACCTGACCGTTCGTCTG
GAAAAAGCAGCGTCCTACGAAGAAATCAAGAAAGCCATCAAAGCCGCTTCTG
AAGGAAATTGTTATCCGCCTTCACAAAA

rpoB:

GCGTTGGCCTGGTACGTGTAGAGCGTGCGGTGAAAGAGCGTCTGTCTCTTGGC
GATCTGGATACCCTGATGCCTCAGGATATGATCAACGCCAAGCCGATTTCCGC
AGCAGTGAAAGAGTTCTTTGGTTCCAGCCAGCTGTCTCAGTTTATGGACCAGA
ACAACCCGCTGTCTGAGATTACGCACAAACGTCGTATCTCCGCACTCGGCCCA
GGCGGTCTGACCCGTGAGCGCGCAGGCTTCGAAGTTCGAGACGTACACCCGAC
CCACTACGGTCGCGTATGTCCGATCGAAACGCCTGAAGGTCCGAACATCGGTC
TGATTAACCTCCCTGTCCGTGTACGCGCAGACCAACGAATATGGCTTCCTTGAGA
CGCCGTATCGTAAAGTGACCGACGGTGTGGTTACTGACGAAATTCACTACCTG
TCTGCTATCGAAGAAGGCAACTACGTTATCGCTCAGGCGAACTCCAACCTGGA
TGAAAACGGCCACTTCGTAGAAGATCTGGTTACCTGCCGTAGCAAAGGCGAAT
CCAGCTTGTTTCAGCCGCGACCAGGTTGACTACATGGACGTATCCACCCAGCAG
GTGGTATCCGTCCGTGCGTCCCTGATCCCGTTCCCTGGAACACGATGACGCCAA
CCGTGCATTGATGGGTGCGAACATGCAACGTCAGGCGGTTCCGACTCTGCGCG
CTGATAAGCCGCTGGTTGGTACCGGTATGGAACGTGCTGTTGCCGTTGACTCCG
GTGTTACTGCCGTGGCTAAACGTGGCGGTACCGTTCAGTACGTGGATGCTTCCC
GTATCGTTATCAAAGTTAACGAAGACGAGATGTACCCGGGCGAAGCAGGTATC
GACATCTATAACCTGACCAAGTACACCCGTTCTAACCAGAACACCTGCATCAA
CCAGATGCCTTGCGTGTCCCTGGGCGAACCTATTGAGCGCGGGCGACGTGCTGG
CAGACGGCCCGTCCACCGACCTCGGTGAGCTGGCGCTGGGTGAGAATGCGT
GTAGCGTTCATGCCGTGGAACGGTTACAACTTCGAGACTCAATTATTNCTCCCC
TCCAAAAAAAAA

tonB:

AGCCTTCTCCGACGCNGCCGATAGAGAANNNNNTGNNGGCTCANAAACGATC
TTGAGCCGCCTCCGGCGGCGCAGCCTGTCTGTGGAGCCCGTTGTTGAACCCGAA
CCTGAGCCGGAGCCAGAGGTAGCGCCTGAACCGCCGAAAGAGGCGCCGGTGG
TGATCCATAAACCGGAACCTAAGCCGAAGCCCAAACCTAAACCCAAGCCTAAG
CCGGAGAAAAAGGTTGAACAGCCGAAGCGGGAAGTGAAGCCGGCAGCAGAGC
CGCGTCCGGCCTCGCCGTTTGAAAACAACATTACGGCGCCGGCGCGTACAGCG
CCAAGTACCTCGACCGCAGCGGCTAAACCCACCGTTACTGCTCCGAGCGGCCC
GCGGGCGATCAGCCGCGTTCAGCCGTCCTATCCGCCGCGCGCTCAGGCGCTGC
GCATTGAAGGGACGGTACGGGTGAAGTTTGACGTTTCGCCTGATGGCCGCATT
GATAATCTGCAGATCCTCTCTGCCAGCCGGCGAATGAAATTGTTATCGGGCCT
CACAAAAA

7. KP9

gapA:

NTCGTTTCGACGGTACCGTTGAAGTGAAAGACGGTCATCTGATCGTTAACGGT
AAAAAAATCCGTGTTACCGCTGAACGTGACCCGGCTAACCTGAAGTGGGACGA
AGTTGGTGTTGACGTTGTTGCTGAAGCAACCGGTATCTTCCTGACCGACGAAA

CCGCTCGTAAACACATCACCGCTGGCGCGAAAAAGTCGTTCTGACTGGCCCG
TCCAAAGACAACACTCCGATGTTCGTTTCGCGGCGCTAACTTCGACGCTTACGCT
GGCCAGGACATCGTTTCCAACGCTTCCTGCACCACCAACTGCCTGGCGCCGCT
GGCTAAAGTTATCAACGACAACCTTCGGTATCGTTGAAGGCCTGATGACCACCG
TCCACGCTACCACCGCTACTCAGAAAACCGTTGATGGCCCGTCTCACAAAGAC
TGGCGCGGCGGCCGCGGCGCAGCTCAGAACATCATCCCGTCTCTACCGGCGC
TGCTAAAGCAGTAGGTAAAGTACTGCCAGAACTGAACGGCAAACCTGACCGGT
ATGGCGTTCCGCGTTCCGACTCCGAACGTATCTGTTGTTGACCTGACCGTTCGT
CTGGAAAAAGCAGCGTCCTACGAAGAAATCAAGAAAGCCATCAAAGCCGCTT
CTGAAGGAAATTGTTANCCCCCTTCAACAAA

rpoB:

GCGTTGGCCTGGTACGTGTAGAGCGTGCGGTGAAAGAGCGTCTGTCTCTTGGC
GATCTGGATACCCTGATGCCTCAGGATATGATCAACGCCAAGCCGATTTCCGC
AGCAGTGAAAGAGTTCTTTGGTTCCAGCCAGCTGTCTCAGTTTATGGACCAGA
ACAACCCGCTGTCTGAGATTACGCACAAACGTCGTATCTCCGCACTCGGCCCA
GGCGGTCTGACCCGTGAGCGCGCAGGCTTCGAAGTTCGAGACGTACACCCGAC
CCACTACGGTCGCGTATGTCCGATCGAAACGCCTGAAGGTCCGAACATCGGTC
TGATTAACCTCCCTGTCCGTGTACGCGCAGACCAACGAATATGGCTTCCTTGAGA
CGCCGTATCGTAAAGTGACCAACGGTGTGGTTACTGACGAAATTCACTACCTG
TCTGCTATCGAAGAAGGCAACTACGTTATCGCTCAGGCGAACTCCAACCTGGA
TGAAAACGGCCACTTCGTAGAAGATCTGGTTACCTGCCGTAGCAAAGGCGAAT
CCAGCTTGTTTCAGCCGCGACCAGGTTGACTACATGGACGTATCCACCCAGCAG
GTGGTATCCGTCCGTGCGTCCCTGATCCCGTTCCCTGGAACACGATGACGCCAA
CCGTGCATTGATGGGTGCGAACATGCAACGTCAGGCGGTTCCGACTCTGCGCG
CTGATAAGCCGCTGGTTGGTACCGGTATGGAACGTGCTGTTGCCGTTGACTCCG
GTGTTACTGCCGTGGCTAAACGTGGCGGTACCGTTCAGTACGTGGATGCTTCCC
GTATCGTTATCAAAGTTAACGAAGACGAGATGTACCCGGGCGAAGCAGGTATC
GACATCTATAACCTGACCAAGTACACCCGTTCTAACCAGAACACCTGCATCAA
CCAGATGCCTTGCGTGTCCCTGGGCGAACCTATTGAGCGCGGCGACGTGCTGG
CAGACGGCCCGTCCACCGACCTCGGTGAGCTGGCGCTGGGTCAGAACATGCGT
GTAGCGTTCATGCCGTGGAACGGTTACAACCTTCGAGATCGATTGATTNNCTCNT
CTCCAAAAAAAAG

tonB:

GAACAGCCTTCTCCGACGCAGCCGATAGAGATCACAATGGTGGCGCCGGCCGA
TCTTGAGCCGCCTCCGGCGGCGCAGCCTGTCGTGGAGCCCGTTGTTGAACCCG
AACCTGAGCCGGAGACAGAGGTAGCGCCTGAACCGCCGAAAGAGGCGCCGGT
GGTGATCCATAAACCGGAACCTAAGCCGAAGCCCAAACCTAAACCCAAGCCTA
AGCCGGAGAAAAAGGTTGAACAGCCGAAGCGGGAAGTGAAGCCGGCAGCAG
AGCCGCGTCCGGCCTCGCCGTTTGAAAACAACAATACGGCGCCGGCGCGTACA

GCGCCAAGCACCTCGACAGCAGCGGCTAAACCCACCGTTACTGCTCCAAGCGG
CCCGCGGGCGATCAGCCGCGTTCAGCCGTCCTATCCGGCGCGCGCTCAGGCGC
TGCGCATTGAAGGGACGGTACGGGTGAAGTTTGACGTTTCGCCTGATGGCCGC
ATTGATAATCTGCAGATCCTCTCTGCTCAGCCGGCGAATGAAATTGTTATCGGG
CTCACAAAAA

8. ESKP3

gapA:

CGTTTCGACGGTACCGTTGAAGTGAAAGACGGTCATCTGGTCGTTAACGGTAA
AAAAATCCGTGTTACCGCTGAACGTGACCCGGCTAACCTGAAGTGGGACGAAG
TTGGTGTGACGTTGTTGCTGAAGCAACCGGTATCTTCCTGACCGACGAAACCG
CTCGTAAACACATCACCGCTGGCGCGAAAAAAGTCGTTCTGACTGGCCCGTCC
AAAGACAACACTCCGATGTTTCGTTTCGCGGCGCTAACTTCGACGCTTACGCTGG
CCAGGACATCGTTTCCAACGCTTCCTGCACCACCAACTGCCTGGCGCCGCTGGC
TAAAGTTATCAACGACAACCTTCGGTATCGTTGAAGGCCTGATGACCACCGTCC
ACGCTACCACCGCTACTCAGAAAACCGTTGATGGCCCGTCTCACAAAGACTGG
CGCGGCGGCCGCGGCGCAGCTCAAAACATCATCCCGTCCTCTACCGGCGCTGC
TAAAGCAGTAGGTAAAGTACTGCCAGAACTGAACGGCAAACCTGACCGGTATG
GCGTTCCGCGTTCCAACCTCCGAACGTATCTGTTGTTGACCTGACCGTTCGTCTG
GAAAAAGCAGCGTCCTACGAAGAAATCAAGAAAGCCATCAAAGCCGCTTCTG
AAGGAAATTGTTATCCCCCCTCACAAAAA

rpoB:

CGTTGGCCTGGTACGTGTAGAGCGTGCGGTGAAAGAGCGTCTGTCTCTTGGCG
ATCTGGATACCCTGATGCCTCAGGATATGATCAACGCCAAGCCGATTTCCGCA
GCAGTGAAAGAGTTCTTTGGTTCCAGCCAGCTGTCTCAGTTTATGGACCAGAA
CAACCCGCTGTCTGAGATTACGCACAAACGTCGTATCTCCGCACTCGGCCAG
GCGGTCTGACCCGTGAGCGCGCAGGCTTCGAAGTTCGAGACGTACACCCGACC
CACTACGGTCGCGTATGTCCGATCGAAACGCCTGAAGGTCCGAACATCGGTCT
GATTAACCTCCCTGTCCGTGTACGCGCAGACCAACGAATATGGCTTCCTTGAGA
CGCCGTATCGTAAAGTGACCGACGGTGTGGTACTGACGAAATTCACTACCTG
TCTGCTATCGAAGAAGGCAACTACGTTATCGCTCAGGCGAACTCCAACCTGGA
TGAAAACGGCCACTTCGTAGAAGATCTGGTTACCTGCCGTAGCAAAGGCGAAT
CCAGCTTGTTACGCCGCGACCAGGTTGACTACATGGACGTATCCACCCAGCAG
GTGGTATCCGTCCGTGCGTCCCTGATCCCGTTCCTGGAACACGATGACGCCAA
CCGTGCATTGATGGGTGCGAACATGCAACGTCAGGCGGTTCCGACTCTGCGCG
CTGATAAGCCGCTGGTTGGTACCGGTATGGAACGTGCTGTTGCCGTTGACTCCG
GTGTTACTGCCGTGGCTAAACGTGGCGGTACCGTTCAGTACGTGGATGCTTCCC
GTATCGTTATCAAAGTTAACGAAGACGAGATGTACCCGGGCGAAGCAGGTATC
GACATCTATAACCTGACCAAGTACACCCGTTCTAACCAGAACACCTGCATCAA
CCAGATGCCTTGCGTGTCCCTGGGCGAACCTATTGAGCGCGGCGACGTGCTGG

CAGACGGCCCCGTCCACCGACCTCGGTGAGCTGGCGCTGGGTGAGAACATGCGT
GTAGCGTTCATGCCGTGGAACGGTTACAACCTTCGAGACCNAATNNTTCCCCC
NCTCAAAAAAAAAA

tonB:

GAACAGCCTTCTCCGACGCAGCCGATAGAGATCACAATGGTGGCGCCAGCCGA
TCTTGAGCCGCTCCGGCGGCGCAGCCTGTCGTGGAGCCCGTTGTTGAACCCG
AACCTGAGCCGGAGCCAGAGGTAGCGCCTGAACCGCCGAAAGAGGCGCCGGT
GGTGATCCATAAACCGGAACCTAAGCCGAAGCCCAAACCTAAACCCAAGCCTA
AGCCGGAGAAAAAGGTTGAACAGCCGAAGCGGGAAGTGAAGCCGGCAGCTGA
GCCGCGTCCGGCCTCGCCGTTTGAACAACAATACGGCGCCGGCGCGTACAG
CGCCAAGTACCTCGACCGCAGCGGCTAAACCCACCGTTACTGCTCCGAGCGGC
CCGCGGGCGATCAGCCGCGTTCAGCCGTCCTATCCGGCGCGCGCTCAGGCGCT
GCGCATTGAAGGGACGGTACGGGTGAAGTTTGACGTTTCGCCTGATGGCCGCA
TTGATAATCTGCAGATCCTCTCTGCTCAGCCGGCGAATGAAATTGTTATCGGCC
TCACAAAA

9. KP10

gapA:

TTTCGACGGTACCGTTGAAGTGAAAGACGGTCATCTGGTCGTTAACGGTAAAA
AAATCCGTGTTACCGCTGAACGTGACCCGGCTAACCTGAAGTGGGACGAAGTT
GGTGTGACGTTGTTGCTGAAGCAACCGGTATCTTCCTGACCGACGAAACCGC
TCGTAAACACATCACCGCTGGCGCGAAAAAAGTCGTTCTGACTGGCCCGTCCA
AAGACAACACTCCGATGTTTCGTCGCGGCGCTAACTTCGACGCTTACGCTGGC
CAGGACATCGTTTCCAACGCTTCCTGCACCACCAACTGCCTGGCGCCGCTGGCT
AAAGTTATCAACGACAACCTTCGGTATCGTTGAAGGCCTGATGACCACCGTCCA
CGTACCACCGCTACTCAGAAAACCGTTGATGGCCCGTCTCACAAGACTGGC
GCGGCGGCCGCGGCGCAGCTCAGAACATCATCCCGTCCTCTACCGGCGCTGCT
AAAGCAGTAGGTAAAGTACTGCCAGAACTGAACGGCAAACCTGACCGGTATGG
CGTTCCGCGTTCCAACCTCCGAACGTATCTGTTGTTGACCTGACCGTTCGTCTGG
AAAAAGCAGCGTCCTACGAAGAAATCAAGAAAGCCATCAAAGCCGCTTCTGA
AGGAAATTGTTATCCGCCTCACAAAAA

rpoB:

GCGTTGGCCTGGTACGTGTAGAGCGTGCGGTGAAAGAGCGTCTGTCTCTTGGC
GATCTGGATACCCTGATGCCTCAGGATATGATCAACGCCAAGCCGATTTCCGC
AGCAGTGAAAGAGTTCTTTGGTTCCAGCCAGCTGTCTCAGTTTATGGACCAGA
ACAACCCGCTGTCTGAGATTACGCACAAACGTCGTATCTCCGCACTCGGCCCA
GGCGGTCTGACCCGTGAGCGCGCAGGCTTCGAAGTTCGAGACGTACACCCGAC
CCACTACGGTCGCGTATGTCCGATCGAAACGCCTGAAGGTCCGAACATCGGTC
TGATTAACCTCCCTGTCCGTGTACGCGCAGACCAACGAATATGGCTTCCTTGAGA

CGCCGTATCGTAAAGTGACCGACGGTGTGGTTACTGACGAAATTCACTACCTG
TCTGCTATCGAAGAAGGCAACTACGTTATCGCTCAGGCGAACTCCAACCTGGA
TGAAAACGGCCACTTCGTAGAAGATCTGGTTACCTGCCGTAGCAAAGGCGAAT
CCAGCTTGTTTCAGCCGCGACCGAGGTGACTACATGGACGTATCCACCCAGCAG
GTGGTATCCGTCGGTGCGTCCCTGATCCCGTTCCTGGAACACGATGACGCCAA
CCGTGCATTGATGGGTGCGAACATGCAACGTCAGGCGGTTCCGACTCTGCGCG
CTGATAAGCCGCTGGTTGGTACCGGTATGGAACGTGCTGTTGCCGTTGACTCCG
GTGTTACTGCCGTGGCTAAACGTGGCGGTACCGTTCAGTACGTGGATGCTTCCC
GTATCGTTATCAAAGTTAACGAAGACGAGATGTACCCGGGCGAAGCAGGTATC
GACATCTATAACCTGACCAAGTACACCCGTTCTAACCAGAACACCTGCATCAA
CCAGATGCCTTGCGTGTCCCTGGGCGAACCTATTGAGCGCGGCGACGTGCTGG
CAGACGGCCCGTCCACCGACCTCGGTGAGCTGGCGCTGGGTCAGAACATGCGT
GTAGCGTTCATGCCGTGGAACGGTTACAACCTTCGAGATCGAATGATTNTCCCC
NNCTCAAAAAAAAAA

tonB:

GAACAGCCTTCTCCGACGCAGCCGATAGAGATCACAATGGTGGCGCCGGCCGA
TCTTGAGCCGCTCCGGCGGCGCAGCCTGTCTGGAGCCCGTTGTTGAACCCG
AACCTGAGCCGGAGCCAGAGGTAGCGCCTGAACCGCCGAAAGAGGCGCCGGT
GGTGATCCATAAACCGGAACCTAAGCCGAAGCCCAAACCTAAACCCAAGCCTA
AGCCGGAGAAAAAGGTTGAACAGCCGAAGCGGGAAGTGAAGCCGGCAGCAG
AGCCGCGTCCGGCCTCGCCGTTTGAAAACAACAATACGGCGCCGGCGCGTACA
GCGCCAAGTACCTCGACCGCAGCGGCTAAACCCACCGTTACTGCTCCGAGCGG
CCCGCGGGCGATCAGCCGCGTTCAGCCGTCCTATCCGGCGCGCGCTCAGGCGC
TGCGCATTGAAGGGACGGTACGGGTGAAGTTTGACGTTTCGCCTGATGGCCGC
ATTGATAATCTGCAGATCCTCTCTGCTCAGCCGGCGAATGAAATTGTTATCGGC
TCACAAAA

10. ESKP4

gapA:

TTTCGACGGTACCGTTGAAGTGAAAGACGGTCATCTGGTCGTTAACGGTAAAA
AAATCCGTGTTACCGCTGAACGTGACCCGGCTAACCTGAAGTGGGACGAAGTT
GGTGTTGACGTTGTTGCTGAAGCAACCGGTATCTTCCTGACCGACGAAACCGC
TCGTAAACACATCACCGCTGGCGCGAAAAAAGTCGTTCTGACTGGCCCGTCCA
AAGACAACACTCCGATGTTTCGTTTCGCGGCGCTAACTTCGACGCTTACGCTGGC
CAGGACATCGTTTCCAACGCTTCCTGCACCACCAACTGCCTGGCGCCGCTGGCT
AAAGTTATCAACGACAACCTTCGGTATCGTTGAAGGCCTGATGACCACCGTCCA
CGCTACCACCGCTACTCAGAAAACCGTTGATGGCCCGTCTCACAAAGACTGGC
GCGGCGGCCGCGGCGCAGCTCAGAACATCATCCCGTCTCTACCGGCGCTGCT
AAAGCAGTAGGTAAAGTACTGCCAGAACTGAACGGCAAACCTGACCGGTATGG
CGTTCCGCGTTCCAACCTCCGAACGTATCTGTTGTTGACCTGACCGTTTCGTCTGG

AAAAAGCAGCGTCCTACGAAGAAATCAAGAAAGCCATCAAAGCCGCTTCTGA
AGGAAATTGTTATCCGCCCTTCACAAAA

rpoB:

CGTTGGCCTGGTACGTGTAGAGCGTGCGGTGAAAGAGCGTCTGTCTCTTGGCG
ATCTGGATACCCTGATGCCTCAGGATATGATCAACGCCAAGCCGATTTCCGCA
GCAGTGAAAGAGTTCTTTGGTTCCAGCCAGCTGTCTCAGTTTATGGACCAGAA
CAACCCGCTGTCTGAGATTACGCACAAACGTCGTATCTCCGCACTCGGCCAG
GCGGTCTGACCCGTGAGCGCGCAGGCTTCGAAGTTCGAGACGTACACCCGACC
CACTACGGTCGCGTATGTCCGATCGAAACGCCTGAAGGTCCGAACATCGGTCT
GATTAACCTCCCTGTCCGTGTACGCGCAGACCAACGAATATGGCTTCCTTGAGA
CGCCGTATCGTAAAGTGACCGACGGTGTGGTTACTGACGAAATTCACTACCTG
TCTGCTATCGAAGAAGGCAACTACGTTATCGCTCAGGCGAACTCCAACCTGGA
TGAAAACGGCCACTTTGTAGAAGATCTGGTTACCTGCCGTAGCAAAGGCGAAT
CCAGCTTGTTTCAGCCGCGACCAGGTTGACTACATGGACGTATCCACCCAGCAG
GTGGTATCCGTCGGTGCGTCCCTGATCCCGTTCCTGGAACACGATGACGCCAA
CCGTGCATTGATGGGTGCGAACATGCAACGTCAGGCGGTTCCGACTCTGCGCG
CTGATAAGCCGCTGGTTGGTACCGGTATGGAACGTGCTGTTGCCGTTGACTCCG
GTGTTACTGCCGTGGCTAAACGTGGCGGTACCGTTCAGTACGTGGATGCTTCCC
GTATCGTTATCAAAGTTAACGAAGACGAGATGTACCCGGGCGAAGCAGGTATC
GACATCTATAACCTGACCAAGTACACCCGTTCTAACCAGAACACCTGCATCAA
CCAGATGCCTTGCGTGTCCCTGGGCGAACCTATTGAGCGCGGCGACGTGCTGG
CAGACGGCCCGTCCACCGACCTCGGTGAGCTGGCGCTGGGTCAGAACATGCGT
GTAGCGTTCATGCCGTGGAACGGTTACACTTCGAGATCAATNATTTTCCCNATCC
ACAAAAAAAAC

tonB:

GAACAGCCTTCTCCGACGCAGCCGATAGAGATCACAATGGTGGCGCCGGCCGA
TCTTGAGCCGCTCCGGCGGCGCAGCCTGTCGTGGAGCCCGTTGTTGAACCCG
AACCTGAGCCGGAGCCAGAGGTAGCGCCTGAACCGCCGAAAGAGGCGCCGGT
GGTGATCCATAAACCGGAACCTAAGCCGAAGCCCAAACCTAAACCCAAGCCTA
AGCCGGAGAAAAAGGTTGAACAGCCTAAGCGGGAAGTGAAGCCGGCAGCAGA
GCCGCGTCCGGCCTCGCCGTTTGAAAACAACAATACGGCGCCGGCGCGTACAG
CGCCAAGTACCTCGACCGCAGCGGCTAAACCCACCGTTACTGCTCCGAGCGGC
CCGCGGGCGATCAGCCGCGTTCAGCCGTCTATCCGGCGCGCGCTCAGGCGCT
GCGCATTGAAGGGACGGTACGGGTGAAGTTTGACGTTTCGCCTGATGGCCGCA
TTGATAATCTGCAGATCCTCTCTGCTCAGCCGGCGAATGAAATTGTTATCGGCC
TCACAAA

11. KP11

gapA:

TTTCGACGAGTACCGTTGAAGTGAAAGACGGTCATCTGGTCGTTAACGGTAAA
AAAATCCGTGTTACCGCTGAACGTGACCCGGCTAACCTGAAGTGGGACGAAGT
TGGTGTGACGTTGTTGCTGAAGCAACCGGTATCTTCCTGACCGACGAAACCG
CTCGTAAACACATCACCGCTGGCGCGAAAAAAGTCGTTCTGACTGGCCCGTCC
AAAGACAACACTCCGATGTTTCGTTTCGCGGCGCTAACTTCGACGCTTACGCTGG
CCAGGACATCGTTTCCAACGCTTCCTGCACCACCAACTGCCTGGCGCCCGTGGC
TAAAGTTATCAACGACAACCTTCGGTATCGTTGAAGGCCTGATGACCACCGTCC
ACGCTACCACCGCTACTCAGAAAACCGTTGATGGCCCGTCTCACAAAGACTGG
CGCGGCGGCCGCGGCGCAGCTCAGAACATCATAACCGTCCTCTACCGGCGCTGC
TAAAGCAGTAGGTAAAGTACTGCCAGAACTGAACGGCAAACCTGACCGGTATG
GCGTTCCGCGTTCCGACTCCGAACGTATCTGTTGTTGACCTGACCGTTTCGTCTG
GAAAAAGCGGCGTCCTACGAAGAAATCAAGAAAGCCATCAAAGCCGCTTCTG
AAGGAAATTGTTATCCGCCTTCACAAAA

rpoB:

GCGTTGGTCTGGTACGTGTAGAGCGTGCGGTGAAAGAGCGTCTGTCTCTTGGC
GATCTGGATACCCTGATGCCTCAGGATATGATCAACGCCAAGCCGATTTCCGC
AGCAGTGAAAGAGTTCTTTGGTTCCAGCCAGCTGTCTCAGTTTATGGACCAGA
ACAACCCGCTGTCTGAGATTACGCACAAACGTCGTATCTCCGCACTCGGCCCA
GGCGGTCTGACCCGTGAGCGCGCAGGCTTCGAAGTTCGAGACGTACACCCGAC
CCACTACGGTCGCGTATGTCCGATCGAAACGCCTGAAGGTCCGAACATCGGTC
TGATTAACCTCCCTGTCCGTGTACGCGCAGACCAACGAATATGGCTTCCTTGAGA
CGCCGTATCGTAAAGTGACCAACGGTGTGGTTACTGACGAAATTCACTACCTG
TCTGCTATCGAAGAAGGCAACTACGTTATCGCTCAGGCGAACTCCAACCTGGA
TGAAAACGGCCACTTCGTAGAAGATCTGGTTACCTGCCGTAGCAAAGGCGAAT
CCAGCTTGTTTCAGCCGCGACCAGGTTGACTACATGGACGTATCCACCCAGCAG
GTGGTATCCGTCGGTGCGTCCCTGATCCCGTTCCTGGAACACGATGACGCCAA
CCGTGCATTGATGGGTGCGAACATGCAACGTCAGGCGGTTCCGACTCTGCGCG
CTGATAAGCCGCTGGTTGGTACCGGTATGGAACGTGCTGTTGCCGTTGACTCCG
GTGTTACTGCCGTGGCTAAACGTGGCGGTACCGTTCAGTACGTGGATGCTTCCC
GTATCGTTATCAAAGTTAACGAAGACGAGATGTACCCGGGCGAAGCAGGTATC
GACATCTATAACCTGACCAAGTACACCCGTTCTAACCAGAACACCTGCATCAA
CCAGATGCCTTGCGTGTCCCTGGGCGAACCTATTGAGCGCGGCGACGTGCTGG
CAGACGGCCCGTCCACCGACCTCGGTGAGCTGGCGCTGGGTGAGAACATGCGT
GTAGCGTTCATGCCGTGGAACGGTTACACTTCGAGANCAATNNTTTNTNCCTCC
ACAAAAAAAAGC

tonB:

AACAGCCTTCTCCGACGCAGCCGATAGAGATCACAATGGTGGCGCCGGCCGAT
CTTGAGCCGCCTCCGGCGGCGCAGCCTGTCGTGGAGCCCGTTGTTGTACCCGA
ACCTGAGCCGGAGCCAGAGGTAGTGCCTGAACCGCCGAAAGAGGCGCCGGTG
GTGATCCATAAACCGGAACCTAAGCCGAAGCCCAAACCTAAACCCAAGCCTAA
GCCGGAGAAAAAGGTTGAACAGCCGAAGCGGGAAGTGAAGCCGGCAGCAGA
GCCGCGTCCGGCCTCGCCGTTTGA AAAACAACAATACGGCGCCGGCGCGTACAG
CGCCAAGTACCTCGACCGCAGCGGCTAAACCCACCGTACTGCTCCGAGCGGC
CCGCGGGCGATCAGCCGCGTTCAGCCGTCCTATCCGGCGCGCGCTCAGGCGCT
GCGCATTGAAGGGACGGTACGGGTGAAGTTTGACGTTTCGCTGATGGCCGCA
TTGATAATCTGCAGATCCTCTCTGCTCAGCCGGCGAATGAAATTGTTATCGGCC
TCACAAAA

12. KP12

gapA:

GTTTCGACGGTACCGTTGAAGTGAAAGACGGTCATCTGGTCGTTAACGGTAAA
AAAATCCGTGTTACCGCTGAACGTGACCCGGCTAACCTGAAGTGGGACGAAGT
TGGTGTTGACGTTGTTGCTGAAGCAACCGGTATCTTCCTGACCGACGAAACCG
CTCGTAAACACATCACCGCTGGCGCGAAAAAAGTCGTTCTGACTGGCCCGTCC
AAAGACAACACTCCGATGTTTCGTTTCGCGGCGCTAACTTCGACGCTTACGCTGG
CCAGGACATCGTTTCCAACGCTTCCTGCACCACCAACTGCCTGGCGCCGCTGGC
TAAAGTTATCAACGACAACCTTCGGTATCGTTGAAGGCCTGATGACCACCGTCC
ACGCTACCACCGCTACTCAGAAAACCGTTGATGGCCCGTCTCACAAAGACTGG
CGCGGCGGCCGCGGCGCAGCTCAGAACATCATCCCGTCCTCTACCGGCGCTGC
TAAAGCAGTAGGTAAAGTACTGCCAGAACTGAACGGCAAACCTGACCGGTATG
GCGTTCCGCGTTCCGACTCCGAACGTATCTGTTGTTGACCTGACCGTTTCGTCTG
GAAAAAGCAGCGTCCTACGAAGAAATCAAGAAAGCCATCAAAGCCGCTTCTG
AAGGAAATTGTTATCCCCCCTCACAAA

rpoB:

CGCGTTGGCCTGGTACGTGTAGAGCGTGCGGTGAAAGAGCGTCTGTCTCTTGG
CGATCTGGATACCCTGATGCCTCAGGATATGATCAACGCCAAGCCGATTTCCG
CAGCAGTGAAAGAGTTCTTTGGTTCCAGCCAGCTGTCTCAGTTTATGGACCAG
AACAACCCGCTGTCTGAGATTACGCACAAACGTCGTATCTCCGCACTCGGCCC
AGGCGGTCTGACCCGTGAGCGCGCAGGCTTCGAAGTTCGAGACGTACACCCGA
CCCACTACGGTCGCGTATGTCCGATCGAAACGCCTGAAGGTCCGAACATCGGT
CTGATTAACTCCCTGTCCGTGTACGCGCAGACCAACGAATATGGCTTCCTTGAG
ACGCCGTATCGTAAAGTGACCGACGGTGTGGTTACTGACGAAATTCACCTACCT
GTCTGCTATCGAAGAAGGCAACTACGTTATCGCTCAGGCGAACTCCAACCTGG
ATGAAAACGGCCACTTCGTAGAAGATCTGGTTACCTGCCGTAGCAAAGGCGAA
TCCAGCTTGTTTCAGCCGCGACCAGGTTGACTACATGGACGTATCCACCCAGCA

GGTGGTATCCGTCGGTGCGTCCCTGATCCCGTTCCTGGAACACGATGACGCCA
ACCGTGCAATTGATGGGTGCGAACATGCAACGTCAGGCGGTTCGACTCTGCGC
GCTGATAAGCCGCTGGTTGGTACCGGTATGGAACGTGCTGTTGCCGTTGACTCC
GGTGTACTGCCGTGGCTAAACGTGGCGGTACCGTTCAGTACGTGGATGCTTCC
CGTATCGTTATCAAAGTTAACGAAGACGAGATGTACCCGGGCGAAGCAGGTAT
CGACATCTATAACCTGACCAAGTACACCCGTTCTAACCAGAACACCTGCATCA
ACCAGATGCCTTGCGTGTCTTGGGCGAACCTATTGAGCGCGGCGACGTGCTG
GCAGACGGCCCGTCCACCGACCTCGGTGAGCTGGCGCTGGGTCAGAACATGCG
TGTAGCGTTTCATGCCGTGGAACGGTTACACTTCGAGACCNAAATTATTTNCTCCC
CCCCAAAAAAGG

tonB:

GCCTTCTCTGACGCNCCCGATGAGAGATNCACAGATGNNGCNGGCTGCACCNAC
CGATCTTGAGCCGCTCCGGCGGCGCAGCCTGTCGTGGAGCCCGTTGTTGAAC
CCGAACCTGAGCCGGAGCCAGAGGTAGTGCCTGAACCGCCGAAAGAGGCGCC
GGTGGTGATCCATAAACCGGAACCTAAGCCGAAGCCCAAACCTAAACCCAAG
CCTAAGCCGGAGAAAAAGGTTGAACAGCCGAAGCGGGAAGTGAAGCCGGCAG
CAGAGCCGCGTCCGGCCTCGCCGTTTGAAAACAACAATACGGCGCCGGCGCGT
ACAGCGCCAAGCACCTCGACAGCAGCGGCTAAACCCACCGTTACTGCTCCAAG
CGGCCCGCGGGCGATCAGCCGCGTTTCAGCCGTCCTATCCGGCGCGCGCTCAGG
CGCTGCGCATAGAAGGTACGGTACGGGTGAAGTTTGACGTTTCGCCTGATGGC
CGCATTGATAATCTGCAGATCCTCTCTGCTCAGCCGGCGAATGAAATTGTTATC
CGGCTCACAAAA

13. KP13

gapA:

GTTTCGACGGTACCGTTGAAGTGAAAGACGGTCATCTGGTCGTTAACGGTAAA
AAAATCCGTGTTACCGCTGAACGTGACCCGGCTAACCTGAAGTGGGACGAAGT
TGGTGTTGACGTTGTTGCTGAAGCAACCGGTATCTTCCTGACCGACGAAACCG
CTCGTAAACACATCACCGCTGGCGCGAAAAAAGTCGTTCTGACTGGCCCGTCC
AAAGACAACACTCCGATGTTTCGTTTCGCGGCGCTAACTTCGACGCTTACGCTGG
CCAGGACATCGTTTCCAACGCTTCCTGCACCACCAACTGCCTGGCGCCGCTGGC
TAAAGTTATCAACGACAACCTTCGGTATCGTTGAAGGCCTGATGACCACCGTCC
ACGCTACCACCGCTACTCAGAAAACCGTTGATGGCCCGTCTCACAAAGACTGG
CGCGGCGGCCGCGGCGCAGCTCAGAACATCATCCCGTCCTCTACCGGCGCTGC
TAAAGCAGTAGGTAAAGTACTGCCAGAACTGAACGGCAAACCTGACCGGTATG
GCGTTCCGCGTTCCAACCTCCGAACGTATCTGTTGTTGACCTGACCGTTTCGTCTG
GAAAAAGCAGCGTCCTACGAAGAAATCAAGAAAGCCATCAAAGCCGCTTCTG
AAGGAAATTGTTATCCCCCTTCACAAAA

rpoB:

GCGTTGGCCTGGTACGTGTAGAGCGTGCGGTGAAAGAGCGTCTGTCTCTTGGC
GATCTGGATACCCTGATGCCTCAGGATATGATCAACGCCAAGCCGATTTCGC
AGCAGTGAAAGAGTTCTTTGGTTCCAGCCAGCTGTCTCAGTTTATGGACCAGA
ACAACCCGCTGTCTGAGATTACGCACAAACGTCGTATCTCCGCACTCGGCCCA
GGCGGTCTGACCCGTGAGCGCGCAGGCTTCGAAGTTCGAGACGTACACCCGAC
CCACTACGGTCGCGTATGTCCGATCGAAACGCCTGAAGGTCCGAACATCGGTC
TGATTAACCTCCCTGTCCGTGTACGCGCAGACCAACGAATATGGCTTCCTTGAGA
CGCCGTATCGTAAAGTGACCGACGGTGTGGTTACTGACGAAATTCCTACCTG
TCTGCTATCGAAGAAGGCAACTACGTTATCGCTCAGGCGAACTCCAACCTGGA
TGAAAACGGCCACTTCGTAGAAGATCTGGTTACCTGCCGTAGCAAAGGCGAAT
CCAGCTTGTTTCAGCCGCGACCAGGTTGACTACATGGACGTATCCACCCAGCAG
GTGGTATCCGTCCGTGCGTCCCTGATCCCGTTCCCTGGAACACGATGACGCCAA
CCGTGCATTGATGGGTGCGAACATGCAACGTCAGGCGGTTCCGACTCTGCGCG
CTGATAAGCCGCTGGTTGGTACCGGTATGGAACGTGCTGTTGCCGTTGACTCCG
GTGTTACTGCCGTGGCTAAACGTGGCGGTACCGTTCAGTACGTGGATGCTTCCC
GTATCGTTATCAAAGTTAACGAAGACGAGATGTACCCGGGCGAAGCAGGTATC
GACATCTATAACCTGACCAAGTACACCCGTTCTAACCAGAACACCTGCATCAA
CCAGATGCCTTGCGTGTCCCTGGGCGAACCTATTGAGCGCGGGCGACGTGCTGG
CAGACGGCCCGTCCACCGACCTCGGTGAGCTGGCGCTGGGTGAGAATGCGT
GTAGCGTTCATGCCGTGGAACGGTTACAACTTCGAGACTCGAANGATTNCCCC
TCCCNAAAAAAAAA

tonB:

AGCCTTCTCCGACGCAGCCGATAGAGATCACAATGGTGGCGCCGGCCGATCTT
GAGCCGCCTCCGGCGGCGCAGCCTGTCGTGGAGCCCGTTGTTGAACCCGAACC
TGAGCCGGAGCCAGAGGTAGCGCCTGAACCGCCGAAAGAGGCGCCGGTGGTG
ATCCATAAACCAGAACCTAAGCCGAAGCCCAAACCTAAACCCAAGCCTAAGCC
GGAGAAAAAGGTTGAACAGCCGAAGCGGGAAGTGAAGCCGGCAGCAGAGCC
GCGTCCGGCCTCGCCGTTTGAACAACAATACGGCGCCGGCGCGTACAGCGC
CAAGTACCTCGACCGCAGCGGCTAAACCCACCGTTACTGCTCCGAGCGGCCCG
CGGGCGATCAGCCGCGTTCAGCCGTCCTATCCGGCGCGCGCTCAGGCGCTGCG
CATTGAAGGGACGGTACGGGTGAAGTTTGACGTTTCGCCTGATGGCCGCATTG
ATAATCTGCAGATCCTCTCTGCTCAGCCGGCGAATGAAATTGTTATCGGCCTCA
CAAAAA

14. *K. aerogenes* – KA1

gapA:

TTTCGACGGTACCGTTGAAGTGAAAGACGGTCATCTGGTCGTTAACGGTAAAA
AAATCCGTGTTACCGCTGAACGTGACCCGGCTAACCTGAAATGGGATGAAGTT
GGTGTGACGTTGTTGCTGAAGCAACCGGTATCTTCCTGACCGACGAAACCGC

TCGTAAACACATCACCGCTGGCGCGAAAAAAGTCGTTCTGACTGGCCCCGTCCA
AAGACAACACTCCGATGTTTCGTTTCGCGGCGCTAACTTCGACACTTACGCAGGC
CAGGACATCGTTTCTAACGCATCCTGCACCACCAACTGCCTGGCTCCGCTGGCT
AAAGTCATCAACGACAACCTTCGGTATCGTTGAAGGCCTGATGACCACCGTTCA
CGCTACTACCGCAACTCAGAAAACCGTTGATGGCCCCGTCTCACAAAGACTGGC
GCGGCGGCCGCGGCGCAGCGCAGAACATCATCCCGTCCTCTACCGGCGCTGCT
AAAGCGGTAGGTAAAGTACTGCCAGAACTGAACGGCAAACCTGACCGGTATGG
CGTTCCGCGTTCCGACTCCGAACGTATCCGTTGTTGACCTGACCGTTCGTCTGG
AAAAAGCAGCGTCCTACGAAGAAATCAAGAAAGCCATCAAAGCCGCTTCTGA
AGGAAATTGTTATCCGGCCTTCAACAAAAA

rpoB:

TCGCGTTGGCCTGGTGCGTGTAGAGCGTGCGGTTAAAGAGCGTCTGTCTCTGG
GCGATCTGGATACCTGATGCCTCAAGATATGATCAACGCCAAGCCGATTTCC
GCAGCAGTGAAAGAGTTCTTTGGTTCCAGCCAGCTGTCCCAGTTTATGGACCA
GAACAACCCGCTGTCTGAGATTACGCACAAACGTCGTATCTCCGCACTCGGCC
CAGGCGGTCTGACCCGTGAACGCGCAGGCTTCGAAGTTCGAGACGTACACCCG
ACTCACTACGGTCGCGTATGTCCAATCGAAACGCCTGAAGGTCCGAACATCGG
TCTGATCAACTCCCTGTCCGTGTACGCACAGACTAACGAATACGGTTTCCTTGA
GACCCCGTATCGTAAAGTGACCGACGGTGTTGTTACCGACGAAATTCATTACC
TGTCTGCTATCGAAGAAGGTAACCTACGTCATTGCTCAGGCGAACTCCAACCTG
GATGACGAAGGCCACTTTGTTGAAGATCTGGTTACCTGCCGTAGTAAAGGCGA
ATCCAGCTTGTTACGCCGCGACCAGGTTGACTACATGGACGTATCCACCCAGC
AGGTGGTATCCGTCGGTGCGTCCCTGATCCCGTTCCTGGAACACGATGACGCC
AACCGTGCAATTGATGGGTGCGAACATGCAACGTCAGGCGGTTCCGACTCTGCG
CGCTGATAAGCCGCTGGTTGGTACCGGTATGGAACGTGCTGTTGCCGTTGACTC
CGGTGTTACTGCGGTAGCTAAACGTGGCGGTACCGTTCAGTACGTGGATGCTT
CCCGTATCGTTATCAAAGTTAACGAAGACGAGATGTACCCGGGCGAAGCAGGT
ATCGACATCTATAACCTGACCAAGTACACCCGTTCTAACCAGAACACCTGCAT
CAACCAGATGCCTTGCGTGTCCCTGGGCGAACCAATTGAACGCGGCGACGTCC
TGGCCGACGGCCCGTCCACCGACCTCGGTGAACTGGCGCTGGGTCAGAACATG
CGCGTAGCGTTCATGCCGTGGAACGGTTACAACCTTCAAGACTCAATNNTTNNN
NCCTCACAAAAAAA

tonB:

AAAGCCTTCGCCTTCACAGCCAATCGAAATCACGATGGTGGCGCCGGCGGATT
TAGAGCCTCCGCAGGCGGCGCAGCCGGTCGTTGAACCCGTTGTTGAACCGGAG
CCGGAACCGGAGCCTGAAGTGGTGCTGAGCCGCCAAAAGAGGCGCCAGTGG
TTATCCACAAACCGGAGCCGAAGCCCAAACCGAAACCAAAGCCTAAGCCGGA
GAAAAAAGTCGAGCCGAAACGCGAGGTGAAGCCCGCTGAGCCGCGCCCGGTC
TCGCCGTTTGAGAATAACAATACGGCACCTGCGCGTACTGCTCCGAGTACCAC

GGCGGCAACGGCAAAACCGATGACCACTGCGCCGAGCGGCCCTAAAGCGTTG
AAACGCGGCGACCCGTCTTACCCGCAACGTGCGCAGGCGCTGCGTATTGAAGG
TGACGTGCGCGTGAAATTTGATGTCACCGCCGATGGTCGTGTGGAGAATATCC
AGATACTCTCCGCCAGCCGGCGAATGAAATTGTTATCGGCCTCACAAAA

15. KP15

gapA:

TTTCGACGGTACCGTTGAAGTGAAAGACGGTCATCTGGTCGTTAACGGTAAAA
AAATCCGTGTTACCGCTGAACGTGACCCGGCTAACCTGAAGTGGGACGAAGTT
GGTGTGACGTTGTTGCTGAAGCAACCGGTATCTTCCTGACCGACGAAACCGC
TCGTAAACACATCACCGCTGGCGCGAAAAAAGTCGTTCTGACTGGCCCGTCCA
AAGACAACACTCCGATGTTTCGTTTCGCGGCGCTAACTTCGACGCTTACGCTGGC
CAGGACATCGTTTCCAACGCTTCCTGCACCACCAACTGCCTGGCGCCGCTGGCT
AAAGTTATCAACGACAACCTTCGGTATCGTTGAAGGCCTGATGACCACCGTCCA
CGCTACCACCGCTACTCAGAAAACCGTTGATGGCCCGTCTCACAAAGACTGGC
GCGGCGGCCGCGGCGCAGCTCAGAACATCATCCCGTCCTCTACCGGCGCTGCT
AAAGCAGTAGGTAAAGTACTGCCAGAACTGAACGGCAAACCTGACCGGTATGG
CGTTCCGCGTTCCGACTCCGAACGTATCTGTTGTTGACCTGACCGTTCGTCTGG
AAAAAGCAGCGTCCTACGAAGAAATCAAGAAAGCCATCAAAGCCGCTTCTGA
AGGAAATTGTTATCGCCCCTNCACAAAA

rpoB:

GCGTTGGCCTGGTACGTGTAGAGCGTGCGGTGAAAGAGCGTCTGTCTCTTGGC
GATCTGGATACCCTGATGCCTCAGGATATGATCAACGCCAAGCCGATTTCGCG
AGCAGTGAAAGAGTTCTTTGGTTCCAGCCAGCTGTCTCAGTTTATGGACCAGA
ACAACCCGCTGTCTGAGATTACGCACAAACGTCGTATCTCCGCACTCGGCCCA
GGCGGTCTGACCCGTGAGCGCGCAGGCTTCGAAGTTCGAGACGTACACCCGAC
CCACTACGGTCGCGTATGTCCGATCGAAACGCCTGAAGGTCCGAACATCGGTC
TGATTAACCTCCCTGTCCGTGTACGCGCAGACCAACGAATATGGCTTCCTTGAGA
CGCCGTATCGTAAAGTGACCAACGGTGTGGTTACTGACGAAATTCACTACCTG
TCTGCTATCGAAGAAGGCAACTACGTTATCGCTCAGGCGAACTCCAACCTGGA
TGAAAACGGCCACTTCGTAGAAGATCTGGTTACCTGCCGTAGCAAAGGCGAAT
CCAGCTTGTTTCAGCCGCGACCAGGTTGACTACATGGACGTATCCACCCAGCAG
GTGGTATCCGTCGGTGCGTCCCTGATCCCGTTCCTGGAACACGATGACGCCAA
CCGTGCATTGATGGGTGCGAACATGCAACGTCAGGCGGTTCCGACTCTGCGCG
CTGATAAGCCGCTGGTTGGTACCGGTATGGAACGTGCTGTTGCCGTTGACTCCG
GTGTTACTGCCGTGGCTAAACGTGGCGGTACCGTTCAGTACGTGGATGCTTCCC
GTATCGTTATCAAAGTTAACGAAGACGAGATGTACCCGGGCGAAGCAGGTATC
GACATCTATAACCTGACCAAGTACACCCGTTCTAACCAAGAACCTGCATCAA
CCAGATGCCTTGCGTGTCCCTGGGCGAACCTATTGAGCGCGGCGACGTGCTGG
CAGACGGCCCGTCCACCGACCTCGGTGAGCTGGCGCTGGGTGAGAACATGCGT

GTAGCGTTCATGCCGTGGAACGGTTACAACCTCCAAGATCGAATGATTTNCCC
CCTCNCAAAAAAAC

tonB:

CAGCCTTCTCCGACGCAGCCGATAGAGATCACAATGGTGGCGCCGGCCGATCT
TGAGCCGCCTCCGGCGGCGCAGCCTGTCGTGGAGCCCGTTGTTGAAACCGAAC
CTGAGCCGGAGCCAGAGGTAGCGCCTGAACCGCCGAAAGAGGCGCCGGTGGT
GATCCATAAACCGGAACCTAAGCCGAAGCCCAAACCTAAACCCAAGCCTAAG
CCGGAGAAAAAGGTTGAACAGCCGAAGCGGGAAGTGAAGCCGGCAGCAGAGC
CGCGTCCGGCCTCGCCGTTTGAACAACAATACGGCGCCGGCGCGTACAGCG
CCAAGTACCTCGACCGCAGCGGCTAAACCCACCGTTACTGCTCCGAGCGGACC
GCGGGCGATCAGCCGCGTTCAGCCGTCCTATCCGCCGCGCGCTCAGGCGCTGC
GCATTGAAGGGACGGTACGGGTGAAGTTTGACGTTTCGCCTGATGGCCGCATT
GATAATCTGCAGATCCTCTCTGCTCAGCCGGCGAATGAAATTGTTATCGGCCTC
ACAAA

16. KP16

gapA:

CGACGGCACCGTTGAAGTGAAAGACGGTCATCTGGTCGTTAACGGTAAAAAA
TCCGTGTTACCGCTGAACGTGACCCGGCTAACCTGAAGTGGGACGAAGTTGGT
GTTGACGTTGTTGCTGAAGCAACCGGTATCTTCCTGACCGACGAAACCGCTCGT
AAACACATCACCGCTGGCGCGAAAAAAGTCGTTCTGACTGGCCCGTCCAAAGA
CAACACCCCGATGTTTCGTTTCGCGGCGCTAACTTCGACACTTACGCTGGCCAGG
ACATCGTTTCCAACGCATCCTGCACTACCAACTGCCTGGCGCCGCTGGCTAAA
GTCATCAACGACAACTTCGGTATCGTTGAAGGCCTGATGACCACCGTCCACGC
TACCACCGCTACCCAGAAAACCGTTGATGGCCCGTCTCACAAAGACTGGCGCG
GCGGCCGCGGCGCAGCTCAGAACATCATCCCGTCTCTACCGGCGCTGCTAAA
GCAGTAGGTAAAGTACTGCCAGAACTGAACGGCAAACCTGACCGGTATGGCGTT
CCGCGTTCCGACTCCGAACGTATCCGTTGTTGACCTGACCGTTCGTCTGGAAAA
AGCAGCGTCCTACGAAGAAATCAAGAAAGCCATCAAAGCCGCTTCTGAAGGA
AATTGTTATCCGCCTCACAAAAAA

rpoB:

CGTTGGCCTGGTACGTGTAGAGCGTGCGGTGAAAGAGCGTCTGTCTCTGGGCG
ATCTGGATACCTGATGCCTCAGGATATGATCAACGCCAAGCCGATTTCCGCA
GCAGTGAAAGAGTTCTTTGGTTCCAGCCAGCTGTCTCAGTTTATGGACCAGAA
CAACCCGCTGTCTGAGATCACGCACAAACGTCGTATCTCCGCACTCGGCCAG
GCGGTCTGACCCGTGAGCGCGCAGGCTTCGAAGTTCGAGACGTACACCCGACT
CACTATGGTCGCGTATGTCCAATCGAAACGCCTGAAGGTCCGAACATCGGTCT
GATCAACTCCCTGTCCGTGTACGCGCAGACCAACGAATATGGCTTCCTTGAGA
CGCCGTATCGTAAAGTGACCGACGGTGTGGTTACTGACGAAATTCACTACCTG

TCTGCTATCGAAGAAGGCAACTACGTTATCGCTCAGGCGAACTCCAACCTGGA
TGAAAACGGCCACTTCGTAGAAGACCTGGTGACCTGCCGTAGCAAAGGCGAAT
CCAGCTTGTTTCAGCCGTGACCAGGTTGACTACATGGACGTATCCACCCAGCAG
GTGGTATCCGTGCGGTGCGTCCCTGATCCCGTTCTGGAACACGATGACGCCAA
CCGTGCATTGATGGGTGCAAACATGCAACGTCAGGCCGTTCCGACTCTGCGCG
CTGATAAGCCGCTGGTTGGTACCGGTATGGAACGTGCTGTGGCCGTCGACTCC
GGTGTACTGCGGTGGCTAAACGTGGCGGTACTGTTTCAGTACGTGGATGCCTC
CCGTATCGTTATCAAAGTTAACGAAGACGAGATGTACCCGGGCGAAGCAGGTA
TCGACATCTATAACCTGACTAAGTACACCCGTTCTAACCAGAACACCTGCATC
AACCAGATGCCTTGCGTGTCCCTGGGCGAACCTATTGAGCGCGGCGACGTGCT
GGCCGACGGCCCGTCCACCGACCTCGGTGAGCTGGCGCTGGGTCAGAACATGC
GCGTAGCGTTTCATGCCGTGGAACGGTTACACTTCAAGACTCAATNTTTTCCCC
CCNAAAACAAAAAAA

tonB:

ACAGCCTTCCCCGACGCAGCCGATAGAGATCACCATGGTGGCGCCGGCCGATC
TTGAGCCGCCCCCGGCGGCGCAACCCGTCGTGCAGCCCGTTGTTGAACCCGAA
CCAGAGCCGGAACCTGAGGTGGTCCCTGAGCCGCCGAAAGAGGCGCCGGTGG
TGATCCATAAACCCAGAACCTAAGCCGAAGCCCAAACCTAAACCGAAGCCCAA
GCCGGAGAAAAAGGTTGAGCAGCCTAAGCGGGACGTCAAGCCGGCAGCGGAG
CCGCGTCCGGCTTCACCGTTTGAAAACAACAATACGACGCCAGCGCGCACCGC
GCCGAGCACCTCCACCGCAGCGGCCAAACCCACCGTCACTGCCCCGAGCGGCC
CGCGGGCCATCAGCCGTGTCCAGCCGACCTATCCGGCGCGCGCTCAGGCGCTG
CGCATTGAAGGGACGGTGCGGGTGAAGTTTGACGTCACGCCAGATGGCCGAAT
TGATAATTTGCAGATCCTCTCTGCTCAGCCGGCGAATGAAATTGTTATCGGCCT
CACAAAAA

17. KP17

gapA:

GTTTCGACGGTACCGTTGAAGTGAAAGACGGTCATCTGGTCGTTAACGGTAAA
AAAATCCGTGTTACCGCTGAACGTGACCCGGCTAACCTGAAGTGGGACGAAGT
TGGTGTTGACGTTGTTGCTGAAGCAACCGGTATCTTCCTGACCGACGAAACCG
CTCGTAAACACATCACCGCTGGCGCGAAAAAAGTCGTTCTGACTGGCCCGTCC
AAAGACAACACTCCGATGTTTCGTTTCGCGGCGCTAACTTCGACGCTTACGCTGG
CCAGGACATCGTTTCCAACGCTTCCTGCACCACCAACTGCCTGGCGCCGCTGGC
TAAAGTTATCAACGACAACCTTCGGTATCGTTGAAGGCCTGATGACCACCGTCC
ACGCTACCACCGCTACTCAGAAAACCGTTGATGGCCCGTCTCACAAAGACTGG
CGCGGCGGCCGCGGCGCAGCTCAGAACATCATCCCGTCCTCTACCGGCGCTGC
TAAAGCAGTAGGTAAAGTACTGCCAGAACTGAACGGCAAACCTGACCGGTATG
GCGTTCGCGTTCGACTCCGAACGTATCTGTTGTTGACCTGACCGTTCGTCTG

GAAAAAGCAGCGTCCTACGAAGAAATCAAGAAAGCCATCAAAGCCGCTTCTG
AAGGAAATTGTTATCCGCCTTCACAAAAAAA

rpoB:

CGTTGGCCTGGTACGTGTAGAGCGTGCGGTGAAAGAGCGTCTGTCTCTTGGCG
ATCTGGATACCCTGATGCCTCAGGATATGATCAACGCCAAGCCGATTTCCGCA
GCAGTGAAAGAGTTCTTTGGTTCCAGCCAGCTGTCTCAGTTTATGGACCAGAA
CAACCCGCTGTCTGAGATTACGCACAAACGTCGTATCTCCGCACTCGGCCAG
GCGGTCTGACCCGTGAGCGCGCAGGCTTCGAAGTTCGAGACGTACACCCGACC
CACTACGGTCGCGTATGTCCGATCGAAACGCCTGAAGGTCCGAACATCGGTCT
GATTAACCTCCCTGTCCGTGTACGCGCAGACCAACGAATATGGCTTCCTTGAGA
CGCCGTATCGTAAAGTGACCAACGGTGTGGTTACTGACGAAATTCACTACCTG
TCTGCTATCGAAGAAGGCAACTACGTTATCGCTCAGGCGAACTCCAACCTGGA
TGAAAACGGCCACTTCGTAGAAGATCTGGTTACCTGCCGTAGCAAAGGCGAAT
CCAGCTTGTTTCAGCCGCGACCAGGTTGACTACATGGACGTATCCACCCAGCAG
GTGGTATCCGTCGGTGCGTCCCTGATCCCGTTCCTGGAACACGATGACGCCAA
CCGTGCATTGATGGGGGCGAACATGCAACGTCAGGCGGTTCCGACTCTGCGCG
CTGATAAGCCGCTGGTTGGTACCGGTATGGAACGTGCTGTTGCCGTTGACTCCG
GTGTTACTGCCGTGGCTAAACGTGGCGGTACCGTTCAGTACGTGGATGCTTCCC
GTATCGTTATCAAAGTTAACGAAGACGAGATGTACCCGGGCGAAGCAGGTATC
GACATCTATAACCTGACCAAGTACACCCGTTCTAACCAGAACACCTGCATCAA
CCAGATGCCTTGCGTGTCCCTGGGCGAACCTATTGAGCGCGGCGACGTGCTGG
CAGACGGCCCGTCCACCGACCTCGGTGAGCTGGCGCTGGGTCAGAACATGCGT
GTAGCGTTCATGCCGTGGAACGGTTACACTTCGAGNTCAATNATTTNCCNNNC
CNAAAAAAA

tonB:

GAACAGCCTTCTCCGACGCAGCCGATAGAGATCACAATGGTGGCGCCGGCCGA
TCTTGAGCCGCTCCGGCGGCGCAGCCTGTCGTGGAGCCCGTTGTTGAACCCG
AACCTGAGCCGGAGCCAGAGGTAGTGCCTGAACCGCCGAAAGAGGCGCCGGT
GGTGATCCATAAACCGGAACCTAAGCCGAAGCCCAAACCTAAACCCAAGCCTA
AGCCGGAGAAAAAGGTTGAACAGCCGAAGCGGGAAGTGAAGCCGGCAGCAG
AGCCGCGTCCGGCCTCGCCGTTTGAAAACAACAATACGGCGCCGGCGCGTACA
GCGCCAAGCACCTCGACAGCAGCGGCTAAACCCACCGTTACTGCTCCAAGCGG
CCCGCGGGCGATCAGCCGCGTTCAGCCGTCCTATCCGGCGCGCGCTCAGGCGC
TGCGCATAGAAGGTACGGTACGGGTGAAGTTTGACGTTTCGCCTGATGGCCGC
ATTGATAATCTGCAGATCCTCTCTGCTCAGCCGGCGAATGAAATTGTTATCGGG
CCTCACAAAAA

18. KP14

gapA:

CGACGGTACCGTTGAAGTGAAAGACGGTCATCTGGTCGTTAACGGTAAAAAA
TCCGTGTTACCGCTGAACGTGACCCGGCTAACCTGAAGTGGGACGAAGTTGGT
GTTGACGTTGTTGCTGAAGCAACCGGTATCTTCCTGACCGACGAAACCGCTCGT
AAACACATCACCGCTGGCGCGAAAAAAGTCGTTCTGACTGGCCCGTCCAAAGA
CAACACTCCGATGTTTCGTTTCGCGGCGCTAACTTCGACGCTTACGCTGGCCAGG
ACATCGTTTCCAACGCTTCCTGCACCACCAACTGCCTGGCGCCGCTGGCTAAAG
TTATCAACGACAACCTTCGGTATCGTTGAAGGCCTGATGACCACCGTCCACGCT
ACCACCGCTACTCAGAAAACCGTTGATGGCCCGTCTCACAAAGACTGGCGCGG
CGGCCGCGGCGCAGCTCAGAACATCATCCCGTCCTCTACCGGCGCTGCTAAAG
CAGTAGGTAAAGTACTGCCAGAACTGAACGGCAAACCTGACCGGTATGGCGTTC
CGCGTTCCGACTCCGAACGTATCTGTTGTTGACCTGACCGTTCGTCTGGAAAAA
GCAGCGTCTACGAAGAAATCAAGAAAGCCATCAAAGCCGCTTCTGAAGGAA
ATTGTTATCCGCCTTCACAAAAA

rpoB:

CGTTGGCCTGGTACGTGTAGAGCGTGCGGTGAAAGAGCGTCTGTCTCTTGGCG
ATCTGGATACCTGATGCCTCAGGATATGATCAACGCCAAGCCGATTTCCGCA
GCAGTGAAAGAGTTCTTTGGTTCCAGCCAGCTGTCTCAGTTTATGGACCAGAA
CAACCCGCTGTCTGAGATTACGCACAAACGTTCGTATCTCCGCACTCGGCCAG
GCGGTCTGACCCGTGAGCGCGCAGGTTTCGAAGTTCGAGACGTACACCCGACC
CACTACGGTCGCGTATGTCCGATCGAAACGCCTGAAGGTCCGAACATCGGTCT
GATTAACCTCCCTGTCCGTGTACGCGCAGACCAACGAATATGGCTTCCTTGAGA
CGCCGTATCGTAAAGTGACCAACGGTGTGGTTACTGACGAAATTCACTACCTG
TCTGCTATCGAAGAAGGCAACTACGTTATCGCTCAGGCGAACTCCAACCTGGA
TGAAAACGGCCACTTCGTAGAAGATCTGGTTACCTGCCGTAGCAAAGGCGAAT
CCAGCTTGTTTCAGCCGCGACCAGGTTGACTACATGGACGTATCCACCCAGCAG
GTGGTATCCGTCGGTTCGCTCCCTGATCCCGTTCCTGGAACACGATGACGCCAA
CCGTGCATTGATGGGTGCGAACATGCAACGTCAGGCGGTTCCGACTCTGCGCG
CTGATAAGCCGCTGGTTGGTACCGGTATGGAACGTGCTGTTGCCGTTGACTCCG
GTGTTACTGCCGTGGCTAAACGTGGCGGTACCGTTCAGTACGTGGATGCTTCCC
GTATCGTTATCAAAGTTAACGAAGACGAGATGTACCCGGGCGAAGCAGGTATC
GACATCTATAACCTGACCAAGTACACCCGTTCTAACCAGAACACCTGCATCAA
CCAGATGCCTTGCGTGTCCCTGGGCGAACCTATTGAGCGCGGCGACGTGCTGG
CAGACGGCCCGTCCACCGACCTCGGTGAGCTGGCGCTGGGTGAGAACATGCGT
GTAGCGTTCATGCCGTGGAACGGTTACAACCTTCGAGATCGAATNTTTTCCCCTC
CCCCAAAAAAA

tonB:

CAGCCTTCTCCGACGCAGCCGATAGAGATCACAATGGTGGCGCCGGCCGATCT
TGAGCCGCCTCCGGCGGCGCAGCCTGTCGTGGAGCCCGTTGTTGAACCCGAAC
CTGAGCCGGAGCCAGAGGTAGCGCCTGAACCGCCGAAAGAGGCGTCGGTGGT
GATCCATAAAACCGGAACCTAAGCCGAAGCCCAAACCTAAACCCAAGCCTAAG
CCGGAGAAAAAGGTTGAACAGCCGAAGCGGGAAGTGAAGCCGGCAGCAGAGC
CGCGTCCGGCCTCGCCGTTTGAAAAACAACAATACGGCGCCGGCGCGTACAGCG
CCAAGCACCTCGACAGCAGCGGCTAAACCCACCGTTACTGCTCCAAGCGGCCC
GCGGGCGATCAGCCGCGTTCAGCCGTCTATCCGCCGCGCGCTCAGGCGCTGC
GCATTGAAGGGACGGTACGGGTGAAGTTTGACGTTTCGCCTGATGGCCGCATT
GATAATCTGCAGATCCTCTCTGCTCAGCCGGCGAATGAAATTGTTATCGGCCTC
ACAAAAA

19. KP5

gapA:

CGACGGTACCGTTGAAGTGAAAGACGGTCATCTGATCGTTAACGGTAAAAAAA
TCCGTGTTACCGCTGAACGTGACCCGGCTAACCTGAAGTGGGACGAAGTTGGT
GTTGACGTTGTTGCTGAAGCAACCGGTATCTTCCTGACCGACGAAACCGCTCGT
AAACACATCACCGCTGGCGCGAAAAAAGTCGTTCTGACTGGCCCGTCCAAAGA
CAACACTCCGATGTTTCGTTTCGCGGCGCTAACTTCGACGCTTACGCTGGCCAGG
ACATCGTTTCCAACGCTTCCTGCACCACCAACTGCCTGGCGCCGCTGGCTAAAG
TTATCAACGACAACCTTCGGTATCGTTGAAGGCCTGATGACCACCGTCCACGCT
ACCACCGCTACTCAGAAAACCGTTGATGGCCCGTCTCACAAAGACTGGCGCGG
CGGCCGCGGCGCAGCTCAGAACATCATCCCGTCCTCTACCGGCGCTGCTAAAG
CAGTAGGTAAAGTACTGCCAGAACTGAACGGCAAACCTGACCGGTATGGCGTTC
CGCGTTCCGACTCCGAACGTATCTGTTGTTGACCTGACCGTTCGTCTGGAAAAA
GCAGCGTCTACGAAGAAATCAAGAAAGCCATCAAAGCCGCTTCTGAAGGAA
ATTGTTATCCGGCCTCACAAAAA

rpoB:

GCGTTGGCCTGGTACGTGTAGAGCGTGCGGTGAAAGAGCGTCTGTCTCTTGGC
GATCTGGATACCCTGATGCCTCAGGATATGATCAACGCCAAGCCGATTTCCGC
AGCAGTGAAAGAGTTCTTTGGTTCCAGCCAGCTGTCTCAGTTTATGGACCAGA
ACAACCCGCTGTCTGAGATTACGCACAAACGTCGTATCTCCGCACTCGGCCCA
GGCGGTCTGACCCGTGAGCGCGCAGGCTTCGAAGTTCGAGACGTACACCCGAC
CCACTACGGTCGCGTATGTCCGATCGAAACGCCTGAAGGTCCGAACATCGGTC
TGATTAACCTCCCTGTCCGTGTACGCGCAGACCAACGAATATGGCTTCCTTGAGA
CGCCGTATCGTAAAGTGACCAACGGTGTGGTACTGACGAAATTCCTACCTG
TCTGCTATCGAAGAAGGCAACTACGTTATCGCTCAGGCGAACTCCAACCTGGA
TGAAAACGGCCACTTCGTAGAAGATCTGGTTACCTGCCGTAGCAAAGGCGAAT
CCAGCTTGTTTCAGCCGCGACCAGGTTGACTACATGGACGTATCCACCCAGCAG

GTGGTATCCGTCGGTGCGTCCCTGATCCCGTTCCTGGAACACGATGACGCCAA
CCGTGCATTGATGGGTGCGAACATGCAACGTCAGGCGGTTCCGACTCTGCGCG
CTGATAAGCCGCTGGTTGGTACCGGTATGGAACGTGCTGTTGCCGTTGACTCCG
GTGTTACTGCCGTGGCTAAACGTGGCGGTACCGTTCAGTACGTGGATGCTTCCC
GTATCGTTATCAAAGTTAACGAAGACGAGATGTACCCGGGCGAAGCAGGTATC
GACATCTATAACCTGACCAAGTACACCCGTTCTAACCAGAACACCTGCATCAA
CCAGATGCCTTGCGTGTCCCTGGGCGAACCTATTGAGCGCGGCGACGTGCTGG
CAGACGGCCCGTCCACCGACCTCGGTGAGCTGGCGCTGGGTCAGAACATGCGT
GTAGCGTTCATGCCGTGGAACGGTTACACTTCGAGACNAATGTTTTCCCCCCCC
NCNAAAAAA

tonB:

GCCTTCTCCGACGCACCCGATAGAGAACNNAATGTNGGCTCCCNANCGATCTT
GAGCCGCCTCCGGCGGCGCAGCCTGTCGTGGAGCCCGTTGTTGAACCCGAACC
TGAGCCGGAGACAGAGGTAGCGCCTGAACCGCCGAAAGAGGCGCCGGTGTTG
ATCCATAAACCGGAACCTAAGCCGAAGCCCAAACCTAAACCCAAGCCTAAGCC
GGAGAAAAAGGTTGAACAGCCGAAGCGGGAAGTGAAGCCGGCAGCAGAGCC
GCGTCCGGCCTCGCCGTTTGAACAACAATAACGGCGCCGGCGCGTACAGCGC
CAAGCACCTCGACAGCAGCGGCTAAACCCACCGTACTGCTCCAAGCGGCCCCG
CGGGCGATCAGCCGCGTTCAGCCGTCCTATCCGGCGCGCGCTCAGGCGCTGCG
CATTGAAGGGACGGTACGGGTGAAGTTTGACGTTTCGCCTGATGGCCGCATTG
ATAATCTGCAGATCCTCTCTGCCAGCCGGCGAATGAAATTGTTATCGGCCTCA
CAAAAA

Appendix V

Phage Structural Protein Sequences

>1st_BASE_3182708_φKPaV03_F.ab1

AGCGGGAATCAGTGACGGGTAGGTGCTGGTTGAGCACGTAGAAGAACACCCA
GTGCATGGATTAATTCTCACTGAGTATGCCGCTGCTCGACTGGACGAGCAGTA
CCTGGGCAACGACGACGAAGAAGAAGTAGAGGAGGCAGCTTAATGTTCTTGA
ATCCGCACGGGATTGATATGCAGCTGCTCTTGCAGATACTGCAAACGCACCGA
CCTAGCTGGGCAAGTACCAAGTGGTTCGAGCCGCTGCTTGAGTGGGCGCTGGG
CAGTGACATGCACTACGTAAAGGACAAGCACGGGAAGTACTTCGTGCTGGTGG
GGGACTCAGAGCAAAGCGACGTAGCGTTTACGTGCGCACCTCGACACGGTGGCC
CGCCCCGGTAGTGACGACCGGACGTTGGTTGCACTAACAAGGGCGTACTGTT
CGTAAAGAATCCGCAGCAGGCTGACTGCTTAGGCGCGGACTGCGGGGCCGGTA
TCTATCTGATGCTGGAGATGCTGCGGCGTGGTGTGCACGGACGTTACTGCTTCT
TCGTGGATGAAGAGGTAGGCTGCGAGGGCAGTGCTGCATCGGTCAAGGATGA
CACTGGGTTTTGGACTGGGGTCAAGGCGATGATTAGCTTTTGACCGGCGCGGT
GACGGTATAATCACGCATCAACGGTACATGCGCTGCTGTTCTGATGCCTTTGCC
AAGACCCTGGCAGAGCGCCTGGGACGCGACGGAGCAGCACTTTGCAGAGGGGG
GTGTATACTGACTCGGCTGATT

>1st_BASE_3195005_φKPaV03_R.ab1

CCGTAATCGGAGACACCATGATGTACTCCGCCGCCTTCGCTGGGTTCTCCTGCA
CCCAGCTAACCAGCTGTTGTTTAGATAGCTGTGACACTACGCGGAACGCAGCC
AGTAACTGCGGGTCCTCGTCCGGCGGCATGTCCACGGCTGCCGTAAACTGAG
CGTAGATGCAGAGAGCCACTGGTCCGGCTCTACTACCTTCGGGTCCCGCTCAA
TCGGAAGGTTGGAGAACGTGCCGTCTTGTAGTACCCGCTCAAGTACCTGGCCC
AGGATGTTTCAGGTCCAGCACCTCATCCGGGGTGTGCTCGTGATGTACCTAC
ACCTACGTTGGTGCACTCATGGATGATGCTAACGAACTCAGCCGAGTCAGTAT
ACACCCCCCTCTGCAAGTGCTGCTCCGTGCGTCCCAGGCGCTCTGCCAGGGTCT
TGGCAAAGGCATCAGAACAGCAGCGCATGTACCGTTGATGCGTGATTATACCG
TCACCGCGCCGGTCAAAGCTAATCATCGCCTTGACCCCAGTCCAAAACCCAGT
GTCATCCTTGACCGATGCAGCACTGCCCTCGCAGCCTACCTCTTCATCCACGAA
GAAGCAGTAACGTCCGTGCACACCACGCCGCAGCATCTCCAGCATCAGATAGA
TACCGGCCCCGCAGTCCGCGCCTAAGCAGTCAGCCTGCTGCGGATTCTTTACG
AACAGTACGCCCTTGTTAGTGCAACCAACGTCCGGTGCTGCACTACCGGGGCG
GGCCACCGTGTCGAGGTGCGACGTAAACGCTACGTCGCTTTGCTCTGAGTCCC
CCACCAGCACGAAGTAGTTCCCGTGCTTGTCTTTACGTAGTGATGTCCTGCTGC
CCAGCGCCCACTCAAGCAGCGGCTCGAACCCTTGGTACTTGCCCAGCTAGGT
CGGTGCGTTTGCAGTATCTGCAAGAGCAGCTGCATATCAATCCCGTGCGGATT

CAAGAACATTAAGCTGCCTCCTNACTTCTCTTCATCGTCTTTGCCAGGTACTGT
TCGTCCATTNAATCANNGGCTTTCTCTGTGAGAATTAATCNATGCCTTGAGGGG
TTCTCTATGTGCCCNTCGNNTCTCCGGGCCCGGG

>1st_BASE_3190206_φKPaV04_T1_F.ab1

GCGGAGTATGCTTGCAATGCTGGCTCCTGATTGGCATGGGAACCATCTGGTCA
CAGTTGTTACAACAGCGTGGGATTTCAAGTTATCAGTGAAAAATTTACTGAGA
TGTTCTGGGAGTTTTTCCACTGAATATCATGCCCGAACCGACGACTTTTGAGG
AGGCCTAAGAAATGTACACTGTTACACACTGCGTCCCTCGAAAATTTTGGGAC
TCTTAGTATCATAATTGGCATTTCCTTGACCAAACCGAAAAACATCGTTTCGA
TCATGATATCTAATTGCTTGGAATAAACTGAACTCCTTCCATTTCCCTACTG
AGAACTGGTAACCCTCAGCGGTCTAATTCTAATTCCCTTGTCTAACAGCATCCT
CGGCTCTCTCTACGCAGATTCTTGAATCAAATCAACACACCCGGAACCATCTG
ATCGCACTCTGGATCCAATTCCTGCGACCATCTGGTCACATTGTTGCGCTATC
TTTTTAAAGTTTCTTTTTTTGCTTTTTTCCATGGTCTCATCC

>1st_BASE_3190207_φKPaV04_T1_R.ab1

ATCGTCCTTATTTCAGTACAGTTCCGGCGGGCCGAGCGTCTGTAGCGGTGATTGC
GGTGGCGATGGGTATGTAGCTATTACAATACTAGTCGTGAAATCGGCCGGAGA
TCAAGTATTATCACATTAAAAGATAAAAAACCAGTGCCTGTGGTTGGTGTCAA
GTCTGTGCATCCTAGTTATTGAACAAGGTCACCTTAGTTGCTTGACGCTAACTA
CCTGATTTAAAGTCTTGTAGATTGTCTTGCTCCCTTCGTTAATTAATCCGCCAAC
GCTCGAGGAGATGCTGGTTGCTTTTTTGGGCACTCAGCCGATTCCCTCATTCACTT
ATTCTCTTCACTCTAGCAGTCTACAACATTAAGCGGTATCAGCTCTCTCTGCTG
TTGCATACGGTTATCCCCCGAATCATGATTTAACACTTTTATAGATCAT

>1st_BASE_3190210_φKPaV08_G23_F.ab1

CTCCGCGCTGTTTACGGTAAAGACCCTCTGGCTGCTGGTGCTAAAGAAGCTTTC
CATCCGATGTACGCTCCGGATGCAATGTTCTCTGGTCAGGGTGCAACCGAGAA
ATTTGAAGCAGTTAAAGCTGGTGGTGCTCTGACTGTTGGTGATATCGTTGTTCA
CGATTTTCGCACAGACCGGTCGTGCTCATCTGCAGGTTGTTGAAGATTTCACTGT
AGACGCTGGTGCTACCGATGCTGCTAACTGGACGCTGCAGTTACTGCCGCTC
TGGAAGCTGGTAAAGTTGTAGAAATCGCCGAAGGTATGGCTACTTCCGTTGCT
GAACTGCAAGAAGCCTTTAACGGTTTCGAAAGATAACCCGTGGAATGAAATGG
GCTTCCGTATCGATAAACAAACCATCGAAGCGAAATCTCGTCAGCTGAAAGCA
CAGTATTCAATCGAACTGGCTCAGGACCTCCGCGCTGTTACGGTATGGACGC
TGATGCTGAACTGAGTTCAATCCTGGCTACCGAAATCATGCTGGAATCCAACC
GCGAA

>1st_BASE_3190211_ϕKPaV08_G23_R.ab1

GCATCAGCGTCCATACCGTGNACAGCGCGGAGGTCCTGAGCCAGTTCGATTGA
ATACTGTGCTTTTCAGCTGACGAGATTTTCGCTTCGATGGTTTGTTTATCGATACG
GAAGCCCATTTCATTCCACGGGTATCTTTTCGAACCGTTAAAGGCTTCTTGCAG
TTCAGCAACGGAAGTAGCCATACCTTCGGCGATTTCTACAACCTTACCAGCTTC
CAGAGCGGCAGTAACTGCAGCGTCCAGTTTAGCAGCATCGGTAGCACCAGCGT
CTACAGTGAAATCTTCAACAACCTGCAGATGAGCACGACCGGTCTGTGCGAAA
TCGTGAACAACGATATCACCAACAGTCAGAGCACCACCAGCTTTAACTGCTTC
AAATTTCTCGGTTGCACCCTGACCAGAGAACATTGCATCCGGAGCGTACATCG
GATGGAAAGCTTCTTTAGCACCAGCAGCCAGAGGGTCTTTACCGTAAACAGCG
CGGAGAGCGAATACCTGACCGGTTGGGTAGTCATCGGCTGAACCCCCCAAAT
ATCAAA

>1st_BASE_3190208_ϕKPaV10_T1_F.ab1

GAAGCCGGTGCTGAATTGCATCTCGTGCACATGACCGCTTAGCGTCATGCATG
CATGCTGCTAGGATGATGCTGTGGAGTTTCTTACCTAACAAGCCTTGAACCTCA
CCTAGTTGCACCTGGGTCTGACAATGGATGCTAAATTTTCGTGAGTGATCAAAC
CCCGAAGAATCTGTTTCAGAACTCGCTCCATATGTTGGAACCTACCCCGGGTTGG
GTAAACTACCACCTTGTTGAGCCAGGTCTGCTTACTTCTTCCACCTGCATCACT
CACCGTAGGTTAGATACCCATTTGCACCCGCATCTGCAACTTGGTCTTTCATGG
CTCCTTGATCCAATAAACTCAGGAACCGCCTGGCACCCCTGTGTCTTGCAGGAT
ACTTGGCCGCTGGTGCTATGCAATCAACTGCAGTATGGATCAGATACAAGTAA
CCCCCTCATCACACA

>1st_BASE_3190209_ϕKPaV10_T1_R.ab1

AGGGAACCAACTTCTCATGCCATACTCGTACAGGTTTTTTGTGTCATTCTCTAT
GTCTAAAGTCCAGTCGTCGTCGTGGTCAAAATTGATCACGAGATTAACCGTCTT
GGAACCATCTGGTCCCCGTATGCAACTGTCTTTCTGTTCTCAGTTTACTTTTATA
GGTCCCTCTAGAGGGAGATTTGATGTATCTAATTTAATTGCGCCAGAGGAATTC
TGATCACATAGAAATGGAACCCTCCGATCTCACACTTAACCAAATTTTCGCATT
GCCCCGGGGGCTGACCGGCGCTTTTCGAGTTAGCGGCAAAATTCCCCTTCGGCC
TTGATGTAGGGTATTTTGTCAAAATAGGAACTAGATTCCCCATGGTCTAAAA
CTTATCAAGCGTTAGAACAAAGCCCTGGTCTTTTGGTTAACACGGTGGAATCCT
TAAATCCCCCACCAGAACTAGGATCTCTTGGATTTCCTAGAAAGCATCCCGCCA
CAGCGAGCGCACCTCTTCAACGAAAATGGACTTGATTTCGTTGGGCGTGCTCAC
GCTTGCGGTTCGATGGGAGAGCCATGCTTGATTTTACGCCCACTAGGCGTGGTCT
GGAACCATCTGGTCGCA

>1st_BASE_3190212_ϕKPaV12_G23_F.ab1

CTCTCCGCGCTGTTTACGGCAAAGACCCGCTGGCTGCTGGTGCTAAAGAAGCT
TTCCATCCGATGTACGCTCCGGATGCAATGTTCTCTGGTCAGGGTGCAACCGAG
AAATTTGAAGCAGTTAAAGCTGGTGCTGCTCTGACTGTTGGTGATATCGTTGTT
CACGATTTTCGCACAGACTGGTCGTGCTCATCTGCAGGTTGTTGAAGNCTTCACT
GTAGACGCTGGTGCTACTGATGCTGCTAAACTGGACGCTGCAGTCACTGCCGC
TCTGGAAGCTGGTAAAGTTGTAGAAATCGCCGAAGGTATGGCTACTTCCGTTG
CTGAACTGCAAGAAGCCTTTAACGGTTCTAAAGATAACCCGTGGAATGAAATG
GGCTTCCGTATCGATAAACTATCGAAGCGAAATCTCGTCAGCTGAAAGC
ACAGTATTCAATCGAACTGGCTCAGGACCTCCGCGCTGTTACGGTATGGACG
CTGATGCTGAACTGAGTTCAATCCTGGCTACCGAAATCATGCTGGAAATTCAA
CCGCGAA

>1st_BASE_3190213_ϕKPaV12_G23_R.ab1

GGANGGCGGGTTGAACTCGCTTGCAGCGTCAGGTCGGCTACGCACTCATCCGT
GGAACGATGCGGNNGTCCCGGATGCTGTGATCGGATACGGTGAATTCCTCT
GACTANNTTTCCTGTCGAAGATTGGTTATCGATAAAGNNNNGNNTNNNTCTNC
TGCTGNTATGTTTGAAACCGTTAANNGCTCCTTGCATTTACCTGCAGTGGTAT
GCNNATCTTCAGCGATTTCCACTACTTCGCCACCTTCTCNACTCTTATGGACTT
CAGCATCCAGTTTAACTGCGTCCAGGACCTCAGCGCCGANTGTGAAGGCTGCT
CCGACGTGGAAGTGAGCTCGATCATGGTGAGCGAAATCATGCTGGAAATCAAC
CGCGAAATCCAGAGCACCACCAGCTTTAACTGCTTCAAATTTCTCGGTTGCACC
CTGACCAGAGAACATTGCATCCGGAGCGTACATCGGATGGAAAGCTTCTTTAG
CACCAGCAGCCAGCGGGTCTTNNCCGTAAACAGCGCGGAGAGCGAAATACCTG
ACCGGTTGGGTTAGTCATCGGCTGAACCCCCCCCCAAAATATCAAA