# CLONING AND EXPRESSION OF PUTATIVE ANTIMICROBIAL CODING GENES FROM PLASMID PMWHK1 OF *PEDOBACTER CRYOCONITIS* STRAIN BG5

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PERPUSTAKAAN UNIVERSITI MALAYSIA SABAH

# THESIS SUBMITTED IN PARTIAL FULFILLMENT FOR THE DEGREE OF MASTER OF SCIENCE

# BIOTECHNOLOGY RESEARCH INSTITUTE UNIVERSITI MALAYSIA SABAH 2018

#### DECLARATIONS

I hereby declare that the material in this thesis is my own except for quotations, excerpts, equations, summaries, and references, which have been duly acknowledged.

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### ABSTRACT

Pedobacter cryoconitis strain BG5 harbours a plasmid, pMWHK1. The circular plasmid consists of 6206 bp nucleotides and eight open reading frames (ORFs) which are deduced as genes. P. cryoconitis BG5 produces antimicrobial compounds that are able to inhibit the growth of food pathogens. However, to date it is not known whether the antimicrobial compounds are conferred by genes in the plasmid or chromosome. There is possibility that the antimicrobial compounds are encoded by genes in plasmid, pMWHK1. Four out of the eight open reading frames were selected in this study which are orf5, orf6, orf7 and orf8. Orf5 is hypothesized to encode for an antitoxin gene whereas orf6, orf7 and orf8 are hypothesized to encode for the antimicrobial gene. The study was conducted to determine if antimicrobial properties of P. cryoconitis BG5 are conferred by orf6, orf7 and orf8 of plasmid pMWHK1. Besides, the study was set-up to determine if the antitoxin gene is encoded by orf5. Another objective of the study is to determine the toxin and antitoxin effect of the genes of plasmid pMWHK1. The putative genes were analysed by expressing them in E. coli. Following expression, the antimicrobial activities and proteins were assayed. Orf5, orf6, orf7 and orf8 were successfully cloned and expressed. Among the ORFs tested, the protein encoded by orf6 was able to inhibit the growth of Bacillus cereus K3 and reduce the growth rate of E. colicarrying that gene. However as tested with expression vectors in E. coli host, orf5 was unable to show effect against orf6 and no toxin-antitoxin effect of the genes were observed. Hence, further study is required as the study may discover new antimicrobial compounds. Findings in this study provided basic foundation or understanding on plasmid pMWHK1.

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#### ABSTRAK

### Pengklonan dan ekspresi andaian gen pengekodan antimikrobial daripada plasmid pMWHK1 dari Pedobacter cryoconitis jenis BG5

Pedobacter cryoconitis jenis BG5 mempunyai plasmid, pMWHK1. Plasmid bulat tersebut terdiri daripada 6206 bp nukleotida dan lapan bingkai bacaan terbuka (ORF). P. cryoconitis BG5 menghasilkan sebatian antimikrob yang dapat menghalang pertumbuhan patogen makanan. Walau bagaimanapun, setakat ini tidak diketahui sama ada sebatian antimikrob tersebut diperoleh daripada gen dalam plasmid atau kromosom. Ada kemungkinan bahawa sebatian antimikrob ini dikodkan oleh gen dalam plasmid, pMWHK1. Empat daripada lapan bingkai bacaan terbuka telah dipilih dalam kajian ini iaitu orf5, orf6, orf7 dan orf8. Orf5 dihipotesis untuk mengkodkan gen antitoksin manakala orf6, orf7 dan orf8 dihipotesis untuk mengkodkan gen antimikrobial. Kajian ini dijalankan untuk menentukan sama ada sifat antimikrobial P. cryoconitis BG5 diberikan oleh orf6, orf7 dan orf8 plasmid pMWHK1. Selain itu, kajian ini juga telah ditubuhkan untuk menentukan sama ada gen antitoxin dikodkan oleh orf5. Objektif lain kajian ini adalah untuk menentukan kesan toksin dan antitoxin gen plasmid pMWHK1. Andaian gen tersebut dianalisis dengan mengekspresikan mereka dalam E. coli. Selepas itu, aktiviti antimikrob dan protein telah diuji. Orf5, orf6, orf7 dan orf8 berjaya diklon dan diekspresikan. Di antara ORF yang diuji, protein yang dikodkan oleh orf6 mampu menghalang pertumbuhan Bacillus cereus K3 dan mengurangkan kadar pertumbuhan E. coli yang membawa gen tersebut. Bagaimanapun, seperti yang diuji dengan vektor ekspresi dalam tuan rumah E. coli, orf5 tidak dapat menunjukkan kesan terhadap orf6 dan tiada kesan toksin-antitoxin daripada gen yang diperhatikan. Oleh itu, kajian lanjut diperlukan kerana kajian tersebut berkemungkinan dapat menemukan sebatian antimikrob baru. Penemuan dalam kajian ini memberikan asas perkara atau pemahaman tentang plasmid DMWHK1.

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# LIST OF ABBREVIATIONS

A <sub>230</sub>	-	spectrophotometric absorbance at wavelength of 230 nm
A <sub>260</sub>	-	spectrophotometric absorbance at wavelength of 260 nm
A <sub>280</sub>	-	spectrophotometric absorbance at wavelength of 280 nm
AMP	-	antimicrobial peptide
Ap <sup>R</sup>	-	ampicillin resistance
<i>ara</i> BAD	8	L-arabinose operon
<i>ara</i> C	2	regulatory protein for P <sub>BAD</sub> promoter
B. cereus	4	Bacillus cereus
bla	-	β-lactamase
BLAST	- 99	Basic Local Alignment Search Tool
bp	-	base pairs
CMV	And all	Cytomegalovirus
DNA	-	Deoxyribonucleic acid
E. cloacae	-	Enterobacter cloacae
E. coli		Escherichia coli
EHEC	-	Enterohemorrhagic <i>E. coli</i>
EK	÷	enterokinase
Exc1	-	entry exclusion protein 1
Gb3	-	glycosphingolipid Gb3
HUS	-	haemolytic uremic syndrome
kb	-	kilobase pairs
kDa	-	kiloDalton

LAB	Э	lactic acid bacteria
MCS	-	multiple cloning site
Mob01	-	mobilization protein
NCBI	-	National Center for Biotechnology Information
nm	-	nanometre
NTS	2	non-typhoidal Salmonella
OD	2	optical density
orf	-	open reading frame
oriV	-	origin of replication
P. cryoconitis	T <sup>a'</sup>	Pedobacter cryoconitis
P <sub>BAD</sub>	<b>.</b>	araBAD promoter
PCR	31	polymerase chain reaction
PSK	<u>*</u>	post-segregational killing
RNA		Ribonucleic acid
S. paratyphi	-A B A	Salmonella paratyphis TI MALAYSIA SABAH
S. typhi	-	Salmonella typhi
SDS-PAGE	•	Sodium dodecyl sulphate-polyacrylamide gel
SIB		Swiss Institute of Bioinformatics
STEC	-	Shiga-toxin producing E. coli
ТА	÷	Toxin-antitoxin
UV	-	ultraviolet
VTEC		Verocytoxin-producing E. coli

# LIST OF SYMBOLS

%		percentage
°C	÷	degree Celsius
μΙ	÷	microliter
μm	-	micrometre
km <sup>2</sup>	1	square kilometre
min		minute(s)
ml	÷	millilitre
mΜ	11	millimolar
ng	-	nanogram
rpm	199	revolutions per minute
S II		second(s)
U	21-22	unit
v	ABA	volt
w/v	-	weight per volume
хg	-	gravitational force
a	-	alpha
β	-	beta

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Dilution at 60 °C

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### **CHAPTER 1**

### INTRODUCTION

#### 1.1 Background

Toxin-antitoxin (TA) systems work by producing a toxin protein that is toxic to the host and also an associated antitoxin (either protein or antisense RNA) that prevent or antagonist to the lethal action of the toxin by proteic complex formation (Van Melderen and De Bast, 2009). In TA systems, the host is not affected by the toxin action when sufficient amount of antitoxin is present to combat the toxin action (Mutschler *et al.*, 2011). The toxin is a stable toxic agent while the antitoxin is an unstable factor so a continuous production of antitoxin is required to prevent the killing of the host cell. The difference of toxin and antitoxin decay rates contributes to plasmid addiction systems where it selectively kills plasmid-free cells (Jensen and Gerdes, 1995).

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An Antarctic bacterium, *Pedobacter cryoconitis* strain BG5 produces antimicrobial compounds that are able to inhibit the growth of food pathogens from five genera namely, *Escherichia* sp., *Salmonella* sp., *Klebsiella* sp., *Enterobacter* sp., and *Bacillus* sp. (Wong *et al.*, 2011). However, to date it is not known whether the antimicrobial compounds are conferred by genes in the plasmid or in the chromosome. There is possibility that the antimicrobial compounds produced to inhibit the growth of food pathogens are encoded by genes in plasmid, pMWHK1. The circular plasmid consists of 6206 bp nucleotides and eight open reading frames (ORFs), named as *orf1*, *orf2*, *orf3*, *orf4*, *orf5*, *orf6*, *orf7*, and *orf8* (Wong *et al.*, 2012). The putative genes *orf1*, *orf7*, and *orf8* encode proteins with unknown function whereas *orf2* and *orf4* encode plasmid mobilization and replication proteins respectively (Wong *et al.*, 2012). Besides, *orf3* gene has been deduced to encode an entry exclusion-like protein that hypothetically to involve in lowering the frequency of DNA transfer via conjugation (Wong *et al.*, 2012). Among the ORFs, *orf5* and *orf6* encode toxin-antitoxin (TA) systems which is hypothesized to confer the plasmid stability and host cell survival (Wong *et al.*, 2012).

The putative genes, *orf5* and *orf6* are selected to be the main highlight of this study as the genes are hypothesized to encode for the antitoxin protein (*pem1*) and toxin protein (*pemk*) respectively. The compound can either be a bacteriocin or protein as the toxin gene *orf6* product is hypothesized to be a *pemK* toxin protein. Apart from that, the antimicrobial compounds could also be the products of putative genes *orf1*, *orf7*, and *orf8* of pMWHK1 that currently known to encode proteins of unknown functions.

Hence, this project is set-out to determine whether any of the ORFs of pMWHK1 encode for the antimicrobial compounds which confer the ability of *P. cryoconitis* strain BG5 to inhibit the growth of food pathogens.

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#### 1.2 Problem Statement

There have been numerous microorganisms isolated from the soil sample of Antarctic including *P. cryoconitis* strain BG5. The bacteria are able to withstand the extreme Antarctic conditions by evolving a few adaptation strategies that are yet to be studied. One of the apparent strategy is BG5 ability to produce antimicrobial compounds probably that allow the bacteria to compete and survive in such conditions.

The compounds are probably encoded in a cryptic plasmid found in *P. cryoconitis* BG5. The plasmid, pMWHK1 contained genes *orf5* and *orf6* which posited to confer the bacteria toxin-antitoxin system. Besides, the products of putative genes of *orf1*, *orf7*, and *orf8* of pMWHK1 are yet to be identified and characterized. The exploitation of pMWHK1 may discover new antimicrobial compounds as the bacteria

showed ability to inhibit the growth of food pathogens and to further understand the adaptation strategies possess by Antarctic bacteria.

This experimental approach will allow the identification and characterization of plasmid pMWHK1 found in *P. cryoconitis* that is isolated from soil of King George Island, Antarctica. However, the difficulties may occurred due to novel compound that may belong to unknown genus or species homology and limited resources of information regarding the compound and bacteria.

Besides, the difficulties may occurred during handling as functional properties of the proteins encoded by the ORFs is unknown. There is also possibility of unknown interaction between the proteins and host molecules such as metabolites, complexes and proteins.

#### 1.3 Hypotheses

The hypotheses of the study are:

- 1. The *orf6*, *orf7* and *orf8* are hypothesized to encode for the antimicrobial genes. The antimicrobial genes can be determined by expressing the ORFs (*orf6*, *orf7* and *orf8*) of plasmid pMWHK1 in an expression vector with *E. coli* host.
- The antitoxin gene is hypothesized to be encoded by *orf5*. The antitoxin gene can be determined by expressing *orf5* of plasmid pMWHK1 in an expression vector with *E. coli* host.

### 1.4 Objectives

The objectives of the study are:

- 1. To determine if antimicrobial properties of *P. cryoconitis* strain BG5 are conferred by the *orf6, orf7* and *orf8* of plasmid pMWHK1.
- 2. To determine if the antitoxin gene is encoded by orf5.
- 3. To determine the toxin and antitoxin effect of the genes of plasmid pMWHK1.



### **CHAPTER 2**

#### LITERATURE REVIEW

#### 2.1 *Pedobacter cryoconitis* strain BG5

Recently, an Antarctic bacteria, *Pedobacter cryoconitis* strain BG5 has been reported by Wong *et al.* (2011) to produce antimicrobial compounds that inhibits the growth of food pathogens from five different genera. Production of the antimicrobial compounds probably provide an advantage to the host to compete for survival against other microorganisms in the same niche for the limited nutrients in the terrestrial environment of the Antarctic (Wong *et al.*, 2011). These antimicrobial compounds can be harnessed for medical applications. In addition, novel antimicrobial compounds may help to combat the multidrug-resistant pathogens. Thus, it will be interesting to discover the types of antimicrobial compounds BG5 produces and also to find out whether the antimicrobial compounds is conferred by the genes of plasmid BG5 harboured.

The optimal growth temperature of *P. cryoconitis* strain BG5 is at 20 °C and tolerably growth at temperature ranging from one to 25 °C (Wong *et al.*, 2012). Besides, *P. cryoconitis* BG5 harbours a cryptic plasmid designated as pMWHK1 (Wong *et al.*, 2012). Interestingly, this psychrophile exhibits multiple resistant towards antibiotics and able to inhibit the growth of food pathogens (Wong *et al.*, 2011).

#### 2.1.1 Morphology and Biochemical Profiles

*P. cryoconitis* BG5 is a Gram-negative bacterium with cell morphology appeared to be spirillum in shape and non-motile (Wong *et al.*, 2011). It showed negative results