UNIVERSITI PUTRA MALAYSIA

ISOLATION AND CHARACTERIZATION OF BACTERIOPHAGE FROM CRAB

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FBSB 2015 93

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Dissertation submitted in partial fulfillment of the requirement for the course BMY 4999 Project in the Department of Microbiology Universiti Putra Malaysia JUNE 2015

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2015

PENGESAHAN

Dengan ini adalah disahkan bahawa projek yang bertajuk "ISOLATION AND CHARACTERIZATION OF BACTERIOPHAGE FROM CRAB" telah disiapkan serta dikemukakan kepada Jabatan Mikrobiologi oleh AMIRA WAHIDA BINTI MOHAMAD SAFIEE (163458) sebagai syarat untuk kursus BMY 4999 projek.

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ABSTRACT

This project was carried out to isolate, purify and characterize the bacteriophage from the crab (*Callinectus sapidus*) also known as blue crab. The crab was obtained freshly from Jetty Wak Sempo, Bagan Lalang, Sepang. Seven bacteria was isolated from the crab and labelled as A, B, C, D, E, F and G were used as a screening agent for the isolation of the bacteriophage. Besides, another 5 species of known bacteria were used for bacteriophage isolation. The bacteria were *Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Bacillus cereus* and *Staphylococcus aureus*. All 12 species of bacteria were used but only bacteria C was infected by the bacteriophage. The bacteriophage was labelled as C-CC. The bacteriophage infected bacteria was identified by gram staining and biochemical tests. Based on the gram staining, the bacterium is a gram negative bacterium with coccobacillus shape. Then, the isolated bacteriophage was characterized based on their protein, genomic, biology and physical characteristics. At the end of the study, the isolated bacteriophage was confirmed classified as DNA-containing bacteriophage with lytic life cycle. The bacteriophage could be as used in the phage therapy against the bacterial contamination.

ABSTRAK

Projek ini dijalankan untuk memencilkan, purifikasi dan menklasifikasikan bakteriofaj daripada ketam (*Callimectus sapidus*) yang juga dikenali sebagai ketam biru. Ketam ini dibeli segar dari Jeti Wak Sempo Bagan Lalang, Sepang. Tujuh bakteria telah dipencilkan daripada ketam dan dilabel sebagai A, B, C, D, E, F dan G dan digunakan sebagai ajen pemeriksaan untuk memencilkan bakteriofaj. Selain itu, terdapat lima bakteria juga yang digunakan untuk memencilkan bakteriofaj. Bakteria-bakteria tersebuat ialah *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, *Bacillus cereus* dan *Staphylococcus aureus*. Kesemua 12 spesis bakteria yang digunakan untuk memecilkan bakteriofaj, hanya bakteria C yang dijangkiti oleh bakteriofaj. Bakteriofaj yang dipencilkan dilabel sebagai bakteriofaj C-CC. Bakteria yang dijangkiti oleh bakteriofaj dianalisis dengan pewarnaan gram dan ujian biokimia. Berdasarkan pewarnaan gram, bakteria C merupakan gram negatif bakteria dan berbentuk coccobacillus. Selepas itu, bakteriofaj yang dipencilkan dianalisa berdasarkan protein, genomik, dan ciri-ciri biologi dan fizikal. Di akhir eksperimen ini, bakteriofaj yang dipencilkan dipastikan diklasifikasikan sebagai DNA-bakteriofaj dengan kitaran hidup lytic. Bakteriofaj juga boleh digunakan sebagai terapi faj terhadap kontaminasi bakteria.

ACKNOWLEDGEMENT

Assalamualaikum W.B.T.

First and foremost I am grateful to Prof. Dr. Tan Wen Siang, my project supervisor. I am extremely thankful and indebted to him for sharing expertise, sincere and valuable guidance, advice and encouragement extended to me. I take this opportunity to express gratitude to the Department of Microbiology and Biochemistry members and staffs for their help, support and for providing equipment and services along my final year project.

I wish to express my sincere thanks to my parents Mohamad Safiee bin Selamat and Fariza binti Ali@Fauzi and family who always give encouragement, moral support and attention to me every time even in a far distance. A million thanks you for the supports and loves.

In addition, my sincere thank you to all the lab seniors in the lab 134, especially to Ms. Wong Chuan Loo. Thank you for helping me in my final year project, giving me a kind guidance, patience and supporting me when I was in troubles.

I take this opportunity to express gratitude to my lab mates which are Farah Hanim binti Agusalim, Nurul Natasya binti Zahari and Najatullah Siddiqi bin Lokman. I am so grateful to have them in the lab. A thousand thanks for sharing valuable information and providing excellent teamwork to me in all the times.

I also place on record, my sense of gratitude to one and all, who directly or indirectly, have lent their hand in my final year project.

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INTRODUCTION

Bacteriophages or phages are viruses that infect bacteria cells and can be found abundantly in environment with an estimation of 10^{32} bacteriophages worldwide (Hanlon, 2007). Bacteriophages are usually found in aqueous environment and have been used as an indicator of bacterial contamination (McLaughlin *et al.*, 2006). The phages' community in the aquatic environment systems was reported by Weinbauer (2004) to be increased in the range of 10^4 to 10^8 virions per ml. They have been highly exploited in many practical applications due to their high specificity in nature (McLaughlin *et al.*, 2007). Recently, phages have been used as a biological control agent to treat human, plant and animal bacterial infections (McLaughlin *et al.*, 2007; Wong *et al.*, 2014; Shivu *et al.*, 2007; Silva *et al.*, 2014).

Asia contributes more than 90% of the world's aquaculture production. Intensive commercialization results in serious diseases problems in the aquaculture sector (Bondad-Reantaso *et al.*, 2005). Food and Agriculture Organization of the United Nations (FAO) (2004) reported that aquaculture industries are the fastest growing food producing sector in the world. The average annual growth rate is 8.9% since 1970, compared to only 1.2% for capture fisheries and 2.8% for terrestrial farmed meat production systems over the same period. Recent statistics indicated that the aquatic sector have reached the production of 9.4% annual percentage growth rate (APR) compared with meat production such as pigs (3.15%), poultry (5.1%), beef and meal (1.2%) and mutton and lamb (1.0%). In 2002, the total world

aquaculture production including aquatic plants was reported to be 51.4 million tonnes by volume and US$ 60.0 billion by value (Crespi, 2005).

Like other farming systems, antibiotics have been frequently used in the aquaculture industries have been frequently used to control or treat the bacteria diseases. However, the extreme usage of antibiotics as therapeutics agent in hatcheries and farms has led to the emergences of antibiotics resistant strains of bacteria in the aquatic environment. Thus, the application of bacteriophages in aquaculture is one of the alternative solutions to curb multi drug-resistant bacteria (Karunasagar *et al.*, 1994). The present study was carried out with the aim to reduce the load of bacteria present in the aquaculture environment through the application of bacteriophage isolated from raw crab (*Callinectus sapidus*).

The objectives of this project were:

1. To isolate and purify the bacteriophage from the crab.
2. To characterize the isolated bacteriophage.

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