UNIVERSITI TEKNOLOGI MARA

ISOLATION AND CHARACTERIZATION OF LYTIC *Enterobacter cloacae* PHAGES ASSOCIATED WITH PAPAYA DIEBACK DISEASE

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ABSTRACT

A search for an effective, green and safe method to control papaya dieback disease (PDD) associated *Enterobacter cloacae* in Malaysia which has resulted in economic losses to farmers motivates this research. Bacteriophages, the natural predator of bacteria could be the solution. Thus, the aims of this study were to isolate and identify lytic phages of *E.cloacae* associated with (PDD), determine their biological properties and stability to exposures to physical and chemical agents and evaluate their suitability as biocontrol agents for *E.cloacae* associated with plant diseases. Their presence was detected by plaque assay using the soft agar overlay method, followed by identification based on Transmission Electron Microscope morphology. The phage nucleic acid type was determined by RNaseA and DNaseI treatment. A one-step growth curve experiment was conducted to ascertain latent period and burst size. The host range was tested against various *E. cloacae* strains, bacterial soft rot of dragon fruits (BSRDF), banana wilt disease (BWD), clinical infections and other phytopathogens. Protein profiles of phages were determined by SDS-PAGE. Based on their latent period, burst size and stability at the different physical conditions their potential as biocontrol agent was evaluated. Of the nine lytic *E. cloacae* phages isolated, five (ECϕ1, ECϕ6, ECϕ7, ECϕ8, and ECϕ9) were further studied. All identified phages were DNA viruses, with ECϕ1, ECϕ8, and ECϕ9 belonging to the *Myoviridae* family, while ECϕ6 and ECϕ7 to the *Inoviridae* family. ECϕ1, ECϕ8, and ECϕ9 exhibited latent periods of 15 to 18 minutes and burst sizes ranging from 41 to 88 particles/cell. ECϕ6 and ECϕ7 had shorter latent periods of six and 12 minutes with burst sizes of two and six particles/cell. The host range of the phages are limited to *E.cloacae* strains from both plants and clinical origins. All the phages except ECϕ6 exhibited thermos-stability up to 60°C. All exhibited pH stability ranging from pH3 to pH10. ECϕ9 was least affected by exposure to UV and chloroform. Based on their protein profiles, two morphotypes were observed in each family. *E.cloacae* phages were successfully isolated and characterized with ECφ9 being evaluated as the most suitable biocontrol agent candidate for *E.cloacae* associated with PPD, BSRDF and BWD as well as with human infections.

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CHAPTER 1
INTRODUCTION

1.1 Research Background

Bacteriophages or phages are viruses that use bacteria as their host cells to replicate by making use of some or all of the host biosynthetic machinery. Like all viruses, phages are metabolically inert in their extra-cellular form, reproducing only after infecting suitable bacterial host. Phages are acknowledged as the most abundant and possibly the most diversified microorganisms on the planet (Pradeep *et al*., 2022; Jurczak-Kurek *et al*., 2016; Mills *et al*., 2013;Kimura *et al*., 2008; Breitbart *et al*., 2002). This diversity is owed to their dynamic adaptation when facing selective pressure such as phage resistance mechanisms,which are widespread in bacterial hosts (Montso *et al*., 2019; Labrie *et al.,* 2010).

Since their discovery more than 100 years ago, phages have been used to biocontrol for bacterial infections and as prophylactic agents. Phages were used as prophylactics against avian typhosis in rural France in 1919 and then were widely used to treat bacterial infections in humansalbeit with a lot of controversies in the pre antibiotic era (Schmidt, 2019). Various studies have now shown that phages have also the potential to treat infections in humans, livestock, plants, andaqua-cultured fish (Guo *et al.*, 2021; Kering *et al*., 2019; Altamirano *et al*., 2019; Fromptom *et al*., 2012; Beaudoin *et al*., 2007; Sulakvelidze *et al*., 2005; Ly- Chatain, 2014; Kutter, 2010) due to their ability to resist various physical conditions in the normal natural conditions. Besides these,the use of phages as a biocontrol agent has been developed, and is rapidly increasing in many fieldssuch as food safety for controlling food and beverage borne pathogens, bioremediation of naturalecosystem, bio sanitization of equipment surfaces in hospitals and those used food production and bio preservation of processed food products with short shelf life (Abdelsattar *et al*., 2021). Phages have also been proved to be a very useful research tools to answer the basic molecular and biophysical questions and have been employed in diagnostics and reporter system,as indicators of water quality and in targeted delivery (Beaudoin *et al*., 2007). The use of phage asa biocontrol agent helps in decreasing the use pesticides and chemicals which could harm the environment, handlers as well as consumers. In addition, the action of bacteriophage