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著者(和文)	ONG SOO PENG
Author(English)	Ong Soo Peng
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論 文 要 旨

THESIS SUMMARY

専攻： Department of	生命理工	専攻	申請学位（専攻分野）： Academic Degree Requested	博士 Doctor of	（Engineering）
学生氏名： Student's Name	Ong Soo Peng		指導教員（主）： Academic Supervisor(main)	丹治保典	
			指導教員（副）： Academic Supervisor(sub)		

要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

This doctoral dissertation is titled "Characterization of *Pseudomonas* lytic phages and their application as a cocktail with antibiotics in controlling *Pseudomonas aeruginosa*", and consists of six chapters.

Chapter 1, General introduction: *P. aeruginosa* is an opportunistic pathogen found among cystic fibrosis (CF) and immunocompromised patients. A high lethal rate in chronic patients is commonly caused by multidrug-resistant (MDR) *P. aeruginosa* after prolonged treatment with antibiotics. Due to emergence of MDR bacteria, phage therapy has gained its attention again from the public in recent years, especially after the successful treatment of diabetic patients from chronic infection. However, *P. aeruginosa* can easily become resistant to phage too. Meanwhile, combination treatment of phage cocktail and antibiotic that target different host mechanism would exert high selective pressure against the bacteria. Reports of using phage cocktail and antibiotics to treat *P. aeruginosa* are scare. Thus, it is important to deepen our understanding about this to ensure a successful treatment.

Chapter 2, Spontaneous mutation leads to antibiotic resistance in *P. aeruginosa*: Combination of different antibiotics is common in treating *P. aeruginosa* due to its intrinsic resistance mechanism. Resistant mutants with several folds increased minimal inhibitory concentration (MIC) appeared in 5^{th} rounds of step wise batch co-culture with increasing concentration of Ciprofloxacin, Meropenem or combination of both. Spontaneous mutations related to Ciprofloxacin and Meropenem resistance were found. Furthermore, spontaneous mutations correspond to both drug's resistance were found to accumulate in strains cultured with both antibiotics.

Chapter 3, Isolation and characterization of phages infectious to *P. aeruginosa*: Phage ΦPA01 and ΦPA02 infectious to *P. aeruginosa* were isolated from wastewater treatment plant in Tokyo. Whole genome sequencing showed that ΦPA01 and ΦPA02 belonged to genus *Pbunalikevirus* and *Phikzlikevirus* respectively. They could infect 36% and 47% of clinical isolates respectively and have different host spectrum, showing their potential to be applied as phage cocktail.

Chapter 4, Identification of host receptor protein: *P. aeruginosa* strains resistant to ΦPA01 or ΦPA02 were generated via batchwise co-culture method. Spontaneous mutations in phage resistant strains were identified by whole genome sequencing. Frameshift mutation which resulted in premature stop codon in *algC* was found in *P. aeruginosa* which was resistant to both ΦPA01 or ΦPA02 (R5-PA01R). Gene *algC* produces

phosphoglucomutase that involves in the synthesis of complete core lipopolysaccharide (LPS). Thus, host receptor protein of ΦPA01 and ΦPA02 was identified as LPS. Knockout of *algC* in *P. aeruginosa* inhibits the infection by both phages. Complementation of *algC* restored infectivity of ΦPA01 and ΦPA02, further supporting that LPS is the host receptor protein of these phages. Furthermore, *P. aeruginosa* which was resistant to ΦPA01 (R4-PA01R) or ΦPA02 (R4-PA02-R) showed different spontaneous mutations, suggesting that *P. aeruginosa* employed different mechanisms to defend against the infection of each phage.

Chapter 5, Synergistic effect of phage and antibiotic: Combination of ΦPA01 and ΦPA02 as phage cocktail could inhibit *P. aeruginosa*'s growth for longer hour (20h) compared to single phage. The phage cocktail was further applied together with Ciprofloxacin or Meropenem and this could inhibit *P. aeruginosa*'s growth up to 96h. Phage propagation was determined in the condition added with antibiotic, showing phages' propagation was not inhibited by Ciprofloxacin or Meropenem. Phage cocktail of ΦPA01 and ΦPA02 showed potential to be used for treatment in *P. aeruginosa*'s infection.

Chapter 6, Conclusion and future prospect: *P. aeruginosa* could gain resistance in a short time when exposed to single or multiple antibiotics through spontaneous mutations. ΦPA01 and ΦPA02 utilizes LPS as host receptor. However, it was suggested that *P. aeruginosa* employed different mechanisms against the infection of ΦPA01 and ΦPA02. Combination of the phage cocktail and Ciprofloxacin or Meropenem as treatment showed prospects to be applied in clinical practice to treat infections caused by *P. aeruginosa*.