INVESTIGATION OF PC1 AND SI BACTERIOPHAGES' PUTATIVE GENOMIC IDENTITIES AND INFECTION METHODS ON PHAGE RESISTANT *PSEUDOMONAS CHLORAPHIS*

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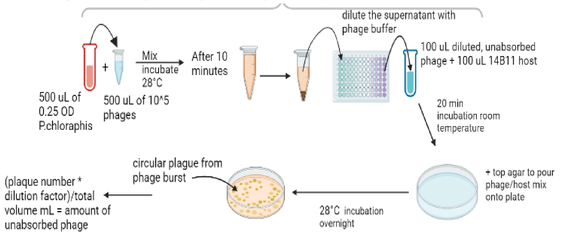
ABSTRACT

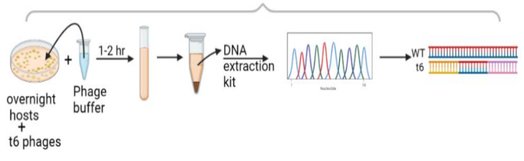
As treatments for infection from drug resistant bacteria become more challenging nowadays, phage therapy arises as an alternative solution. Phage therapy uses phages (viruses that targets bacteria) to kill drug resistant bacteria in infection. The therapy, however, faces difficulties in finding suitable and well-characterized phages to clinically target infections. This thesis aims to enrich the current phage resource by providing some genomic and phenotypic characterization of two isolated phages, PC1 and SI on 14B11 host strain. Several methods were used includes (i) the analysis of phages' infectivity via microbiology assays, (ii) the genomic analysis of two phage resistant hosts against these phages and phage adsorption rate, and (iii) characterization of phage genetics. Subsequently, host range analysis shows that SI infect 3 phage resistant mutants from 14B11, while PC1 does not infect any. PC1 displays a bigger plaque size than SI. Genetic evidence and adsorption assays indicate that PC1 and SI can adsorb into one mutant, suggesting flagella and lipopolysaccharides as potential receptors for SI and PC1, respectively. Phage genomic data suggest PC1 may belong to a T7-like phage groups with similar infection mechanisms as T7 phage, while SI can be a potential new phage species. Mutations in host genome also relate to genes in metabolism and stress response pathways of hosts, suggesting how phages may infect from the inside.

METHODS AND MATERIALS

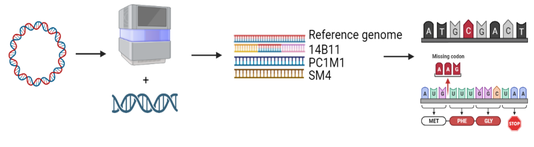
Phage adsorption (kinetic) assay



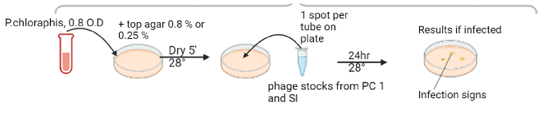
Phage genomic analysis and taxonomy



Mutation analysis of phage resistant genomes



Microbiology assays – spot test and plaque assay



INTRODUCTION

Significance of phage characterization – phage therapy

- Due to the rise of phage resistant bacteria, the used phages are no longer effective and new phage replacement is needed.
- The lack of well studied phages limits the number of therapeutic phage types and also limit using different phages for a better therapy.

Host and phage models

- Therefore, to enrich the phage resource, two different phages, PC1 and SI, were isolated from water and soil respectively and were characterized.
- Knowledge on how PC1 and SI infect hosts, such as phage/host binding mechanisms phages use to kill hosts were studied by looking at what mutations arise in phage resistant bacteria that may contribute to phage resistance.
- The bacterial model is a member of *Pseudomonas* genus, which includes pathogen that cause serious infection in humans
- Two phage resistant mutants were previously created from *Pseudomonas chloraphis* wild type strain 14B11, including PC1M1 being evolved to resist against PC1, and SM4, being subsequently evolved against SI.

GOALS AND HYPPOTHESIS:

1. To elucidate the physiology and genetic traits of PC1 and SI.
2. To find genetic evidence for potential phage receptors and infection mechanisms .

- It is predicted that mutations in phage resistant hosts would affect genes for phage in genes related to biological systems inside hosts, which phages used for reproduction and host lysis.

Phage reproduction cycle

RESULTS

1. Infectivity profile and phenotypes of SI and PC1 on all host strains.

(+) phage infection (-) phage resistance.

Phage resistant mutants	Infection by SI	Infections by PC1
14B11	+	+
PC1M1	+	-
1,2,M1	+	-
1,2,7M	+	-
SM4	-	-

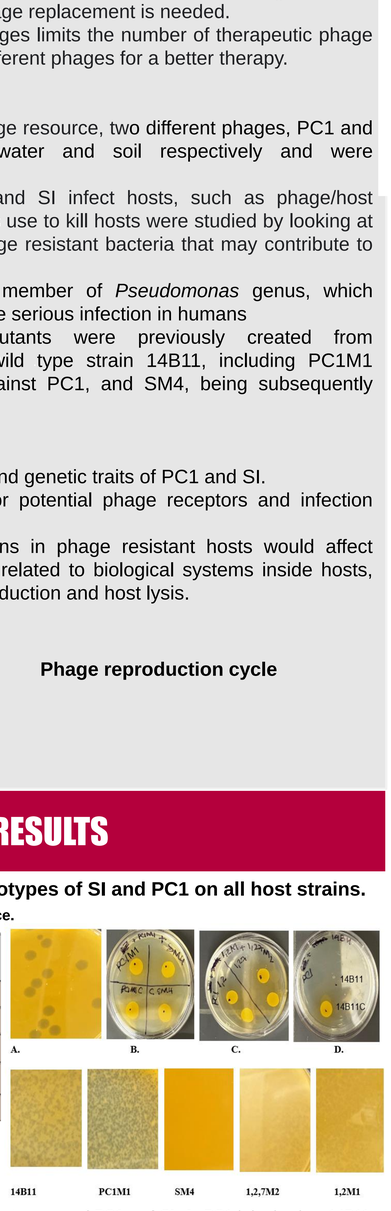


Figure 1. Plaque morphologies and host ranges of PC1 and SI. A. PC1 infection into 14B11 host through plaque assay. B, C Spot test, one mutant spot was treated with phage, and one control spot (no phage, labeled "mutant name C") was displayed. 14B11 spot with PC1 spotted on top was cleared out, while the control spot is intact. PC1 resistance was displayed on all mutants. E. SI infection into 14B11, SM4, and PC1M1 at identical phage and host concentrations, through plaque assay. SI plaque morphologies and virulence on wild type and phage resistant mutants were shown.

→ PC1 and SI carry lytic properties. SI, however, produces turbid plaque on two mutants showed abilities to switch toward lysogenic cycle under special effects from these two mutants, which may not be beneficial

2. Phage resistance primarily occurs on SM4 cell membrane while occurring from the inside of PC1M1

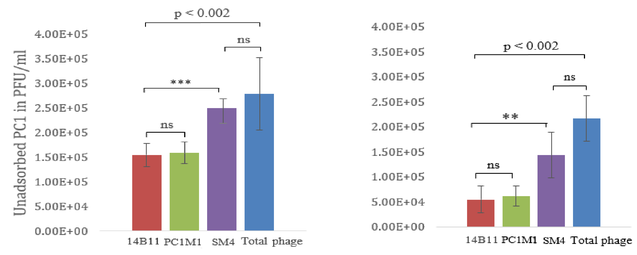


Figure 2. Amount of unadsorped phage (PC1-left and SI – right) into the wildtype and mutant hosts. A. Unadsorped PC1 into all host strains. **B.** unadsorped SI into all host strains. "Total phage" column represents the total amount of phage used in the assay. Error bars represented standard errors. Statistically significant were annotated: ** p < 0.01, *** p <0.001, and ns non-significant.

- Both SI and PC1 effectively adsorped into the wild type and PC1M1, compared to the total phages ($p < 0.002$).

→ Potential modification of PC1 receptor is not sufficient to block PC1 getting inside PC1M1 cells .

- Between the two phages (PC1 and SI), a higher rate of SI adsorption into 14B11 and PC1M1 than PC1 adsorption was reported.
- Both SI and PC1 hardly adsorped into SM4.

→ In SM4, SI and PC1 infection was blocked primarily from the extracellular system in SM4.

3. Phage resistant genomes carry mutations that indicate potential phage receptors and infection mechanisms

Coding mutations
Glycosyltransferase OS assembly cluster (LPS synthesis)
Intergenic mutations
-Cupin family protein (LPS synthesis)/Basic proline-rich - - Protein precursor
FAD-binding protein (oxidoreductase)
***Large scale mutation**
Thioredoxin

PC1M1 SM4

Coding
-*flhA* (flagella biosynthesis protein)
Intergenic
- Glycerophosphoryl diester phosphodiesterase (potential phage resistance protein)
- Protein/DegT/DnrJ/EryC1/Str S aminotransferase (synthesis of secondary metabolites)

Coding
-Wyz (LPS synthesis)
- UDP- GalU (LPS synthesis)
- Efflux pump MexE
Intergenic
-ABC transporter protein ProX/ protein in carnitine cluster (membrane protein/ oxidative stress)
OprO porin protein / hypothetical protein (ion and drug transporter)
Putative lipoprotein/ antitoxin protein (Phage entry blocking / abortive system)

- SM4, which resist against SI, carries mutations in both fhlA and LPS biosynthesis. Adsorption assay and mutation data suggests flagella as a potential receptor for SI.
- Potential relation exists between metabolism and phage reproduction.
- Mutations in thioredoxin, which is responsible for phage assembly

4. Genomic identities of PC1 and SI

Species closely related to PC1	Query coverage	Percentage Identity
Pseudomonas phage phiPsa17	72%	98.56%
Pseudomonas phage WRT	72%	98.32%
Pseudomonas phage Pf1 ERZ-2017	71%	97.30%
Pseudomonas phage PCW2	69%	97.20%
Pseudomonas phage vB_Pci_PCMW57	68%	97.76%
Pseudomonas phage gh-1	53%	99.43%

- Both SI and PC1 are dsDNA phages.
- PC1 potentially belongs to the Ghunavirus family, in the T7 like virus group, which use LPS as phage receptor.
- PC1 resistant hosts carry mutations in LPS synthesis genes while T7 phage also use O-antigen on LPSs as phage receptors.[95] Thus, LPS is a putative receptor for PC1.
- SI carries a genome with 394kbp in length and a GC content of 48.95%. Putative related species have extremely low query coverage to SI genome alignment (3% query coverage, 73%) sequence similarity)

Current Research Question

- Whether if SI and PC1 are broad host range phages for therapy are unclear. Therefore, further testing of SI and PC1 on a larger panel of hosts and what may cause SI's switch toward lysogenic are required.
- Further experiments on individual role of the mutated genes listed here, via gene deletion, mutagenesis, or expression measurement, should be performed to confirm the genes' involvement as phage receptors and phage reproduction.
- Whether if the mutated genes function cooperatively or not to contribute toward phage resistance is unknown.

Conclusion

Given the urgent need to tackle infections from drug resistant bacteria, phage therapy is a rising alternative. To facilitate the process of finding suitable phages for medicine, extensive characterization of phages needs to be done. An enriched phage resource of well-studied phages would provide more choice for phage treatment designs and cure more patients. The phages that were characterized here expand the current phage resource and are promising tools for phage genomic control to target pathogenic infections.

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