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Bacteriophage Isolated from *Burkholderia pseudomallei* Causes Phenotypic Changes in *Burkholderia thailandensis*

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ABSTRACT: *Burkhoderia pseudomallei*, a causative agent of melioidosis, is a gram-negative bacillus that is closely related to its avirulent counterpart, *B. thailandensis*. Previous reports suggested that ninety-two percent of *B. pseudomallei* species carried prophages. In this study, 21 phages isolated from 64 isolates of *B. pseudomallei* were able to lyse some strains of *B. thailandensis* and 3 phages converted *B. thailandensis* to lysogens. By DNase and RNase treatment of the genomes, all these three phages, ϕC2, ϕC13 and ϕC32, were DNA viruses. By nucleic acid type and morphology, ϕC2 and ϕC13 were grouped in the *Myoviridae* family and ϕC32 was in the *Podoviridae* family. The ϕC32 phage described here appears to be the first member of the *Podoviridae* family that was isolated from *Burkholderia* spp. To assess the phenotypic changes of *B. thailandensis* before and after infection with these three phages, the whole-cell protein profile, the resistance to normal human serum (NHS) and the basic biochemical characters were examined and compared. Changes in protein profiles were clearly observed in the T9ϕC2 and T10ϕC2 lysogens when compared with the phage-recipients. In addition, while the T10 recipient was sensitive to serum, both lysogenic T10ϕC2 and its phage-donor C2 grew well in the presence of 30% NHS. There is only one difference in biochemical activities based on the API20NE kit. The phage-recipient T9 could use esculin as an energy source but T9ϕC2 lysogen and the phage-donor C2 was unable to hydrolyse esculin. The results here suggested that changes in phenotypic characters could occur after *B. thailandensis* received the phage from *B. pseudomallei.*

KEYWORDS: bacteriophage, *B. pseudomallei*, *B. thailandensis*, phenotype and protein profile.

INTRODUCTION

Phenotypic characters of bacteria can be controlled by environmental factors and/or genetic codes within the chromosome or plasmid or mobile element of the bacteria. By living together in the same environment, closely related species of bacteria are able to exchange or transfer some genetic materials to their neighbors. Provided that the gene(s) encoded in the acquired genetic factors are expressed, and the gene products involved in functional properties, phenotypic variation can possibly occur.

B. pseudomallei is a small, motile, gram-negative, obligately aerobic, non-spore-forming bacillus that shows a bipolar staining characteristic. It is the causative agent of melioidosis and can be recovered readily from water and wet soil in rice paddy fields in endemic areas, such as Southeast Asia and Northern Australia. Previous studies suggested that *B. pseudomallei* could be distinguished into two types by its ability to utilize the sugar L-arabinose as a sole energy source for growth; arabinose negative (ara^-) and arabinose positive (ara^+)[1]. Only ara^- strains have been isolated from clinical specimens[2]. Later, the ara^+ strain was considered as an avirulent biotype of *B. pseudomallei*, *B. thailandensis*[3]. It is highly likely that these two bacteria, which are closely related species, could be found as a mixed population in the natural environment[4].

Reference cultures of *B. pseudomallei* spontaneously produced bacteriophages at a high frequency, and a certain strain produced at least 2 morphological types of bacteriophages (polylysogeny)[5]. Pure lines of these bacteriophages were isolated and showed a broad bacteriolytic range on reference cultures of *B. pseudomallei*[6]. Grishkina and Merinova, 1993, also established that 40 of 57 studied strains among melioidosis museum cultures were capable of spontaneously producing bacteriophages and all strains were resistant to their own bacteriophages[7]. In Thailand, a trial study confirmed the high percentage of lysogenization among *B. pseudomallei* cultures from soil, animals and clinical specimens[8]. These lysogenic

strains released phages spontaneously and could be detected by co-culturing with other strains or stimulating with mitomycin C. *B. pseudomallei* bacteriophages also allowed a close approach of rapid *B. mallei* identification from other *Burkholderia* species[9,10]. In addition, the bacteriophage, E125, from *B. thailandensis* forms plaques specifically on *B. mallei*. Taken together, the phage of *B. pseudomallei* and *B. thailandensis* may be used as a diagnostic tool for *B. mallei*[11]. However, evidence of phenotypic characteristic changes among *B. mallei* strains after *B. pseudomallei*- and *B. thailandensis*-phage infection, as well as phenotypic changes in *B. thailandensis* after lysogenic induction with the *B. pseudomallei* phage, has not been verified. The aim of this study is to compare protein profiles and other phenotypic characteristics of *B. thailandensis* after infection with bacteriophages from *B. pseudomallei* clinical strains to those of their recipient strains (which were unable to produce that particular phage) and donor strains (which provided the phage particles).

Materials and Methods

Bacteria

The bacterial cultures were from the DMST Culture Collection, National Institute of Health, Thailand and were kindly provided by Assoc. Prof. Prasit Tharavichitkul, Department of Microbiology, Faculty of Medicine, Chiang Mai University. All isolates of *B. pseudomallei* and *B. thailandensis* were confirmed by biochemical tests and stored in 20% (v/v) glycerol suspensions at -70°C.

Induction of Phages from *B. pseudomallei*

The method of phage induction described was modified from Gerhardt et al[12]. Briefly, 75 µl of overnight culture of *B. pseudomallei* was inoculated into 3 ml of tryptic soya broth (TSB) (Merck), which was then incubated at 37°C on a gyratory shaker (250 rpm) (Jeio Tech Co., LTD, South Korea) for 6 h (bacterial cell concentration was approximately 1 x 10^8 to 2 x 10^8 cells/ml). To induce phage, 0.5 µg/ml of mitomycin C (Fluka) was added and shaking continued for 30 min. After that, the culture broth was centrifuged at 7,000 x g at 4°C for 10 min and washed once with sterile normal saline solution. The cells were suspended in 3 ml of pre-warmed TSB broth supplemented with 10 mM $CaCl_2$ (TSB/$CaCl_2$) and culturing continued under shaking conditions at 37°C for 4 h. Phage-containing supernatant was harvested, filtered through a 0.22-µm-pore-size filter, titrated and stored at 4°C.

Detection and Selection of Lysogenic Strains

B. thailandensis was lysogenized with the phage-containing supernatant by the spot testing method[13]. Briefly, approximately 2 ml of 6 h *B. thailandensis* broth culture was poured onto a TSA/$CaCl_2$ plate and the excess fluid was removed. After the plate had been dried, 10 µl of each phage lysate was dropped onto it and incubated at RT overnight. Lysogens were detected when a thin halo of lysis appeared around the spot. The lawn of lysogen was picked, restreaked on the new plate and incubated at 37°C for 1-2 days. The lysogenic strains were kept in 20% (v/v) glycerol and stored at -70°C.

Phage Purification

The phage-containing supernatant was purified by the plaque purification method using successive single-plaque isolation[13] on a propagating or suitable host strain. Molten soft agar containing 10 mM $CaCl_2$ in 2.5 ml were seeded with 200 µl of overnight culture of a suitable host strain and 10 µl of phage-containing supernatant (with a multiplicity of infection (MOI) of 0.05). After mixing, the mixture was poured rapidly onto a TSA/$CaCl_2$ agar plate, swirled and air-dried. The plate was incubated at RT overnight. After incubation, plaques were observed, and a single plaque was picked from the lawn and blown into a tube containing the 200 µl of overnight culture of the same host strain. It was then re-plaqued until uniform plaques were observed.

Phage Propagation and Concentration

Purified phages were propagated using suitable hosts by the broth method modified from Brainbrige[13]. Briefly, 2 ml of overnight culture of a suitable host was inoculated into 200 ml of TSB broth. The cultivation was done at 37°C on a gyratory shaker (250 rpm) for 4-6 h (approximately 10^8 exponentially growing cells/ml). The purified phages were added to a suitable MOI (most to an MOI of 0.001), and $CaCl_2$ was also supplemented at a trial concentration of 10 mM. The shaking was continued until most bacterial cells were lysed or the culture turned clear. Finally, the cell debris was removed by centrifugation for 10 min at 11,000 x g. The clarified supernatant was concentrated with 10% (w/v) polyethylene glycol (PEG) 8000 (Fluka) and 0.5 M sodium chloride (Merck) at 4°C.

Transmission Electron Microscopy (TEM) by Negative Staining with Phosphotungstic Acid (PTA)

To observe phage particles in each lysate, 10 µl of a purified phage sample was dropped fully on a carbon-coated grid lying on paraffin and settled at RT for 30 min. Excess fluid was removed by gently touching under the grid with blotter or filter membrane. Then, 10 µl of 2% (w/v) PTA (pH 7.0) was dropped onto the grid for 10 min in a dark room and excess stain was repelled by taping a filter membrane under the grid. Phage

morphology was examined by TEM (JEM-1200EX II, JEOL, Tokyo, Japan) at an accelerating voltage of 80 kV. Electron micrographs were taken at a magnification of 100,000x. The phage size was determined from the average of three independent measurements.

Protein Profile by Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Whole cells of *B. thailandensis* lysogens, recipient strains, and *B. pseudomallei* donor strains were sonicated at 100 volts 30 sec for six times on ice. The cell lysate proteins were separated on a 15% SDS-polyacrylamide gel and stained with Coomassie blue R-250 (Amersham-Pharmacia, Uppsala, Sweden).

Densitometrical Analysis

The dried gels were analyzed for densities of the protein bands by the Image J program (NIH, Bethesda, MD, U.S.A.) and the results were averaged from three electrophoresis runs.

Resistance to Serum

Bacterial multiplication in 30 % normal human serum (NHS) was assayed on a microtitre plate by a method modified from Ismail et al.[14]. Briefly, the isolated colony of lysogens, and phage-recipient and phage-donor strains were separately inoculated in 2 ml of TSB broth and grown for 6 h at 37°C (approximately 10^8 exponentially growing cells/ml). The culture (10 µl) was added to individual wells in the microtitre plate containing 100 µl of 30% NHS in PBS, pH 7.6. The microtitre plate was then incubated in a rotary shaker (175 rpm) at 37°C for 2 h. The numbers of viable bacteria or colony-forming units present in each well were determined by dropping serial dilutions onto TSA plates and the numbers of colonies were counted after incubation at 37°C for 1-2 days.

Biochemical Test by API 20NE Kit

B. thailandensis lysogens, their phage-recipients, and *B. pseudomallei* phage-donor strains were tested by a commercial biochemical test (API 20NE kit, Biomérieux, Marcy-l' Etoile, France). The reactions were identified by referring to the analytical profile index or using identification software.

Results

Induction of Phages from *B. pseudomallei*

Phage induction of 35 clinical and 29 soil isolates of *B. pseudomallei* was performed by the mitomycin C treatment method. The spot test method described by Chopin et al.[15] was used as an initial test for the presence of phages by lytic activity and plaque formation assay on *B. thailandensis*. It was found that twenty-one (32.81%) of 64 *B. pseudomallei* inducing-lysates formed plaques on 11 of 22 isolates of *B. thailandensis* (data not shown). Sixteen of 21 phage-containing lysates were provided from clinical isolates and the rest were from soil isolations.

The Detection of Successfully Lysogenized *B. thailandensis*

To detect *B. thailandensis* lysogens, phage-containing lysates were spotted onto plates that were flooded with *B. thailandensis*. Successfully lysogenized *B. thailandensis* isolates were selected by the presence of inhibition reactions. Three phages, named ϕC2, ϕC13 and ϕC32, produced inhibition reactions on *B. thailandensis* (4.69%) and resulted in 6 lysogens. Phage ϕC2 formed an inhibition reaction with *B. thailandensis* T9 and T10 strains and the two lysogens were designated as T9ϕC2 and T10ϕC2, respectively. Three lysogens; T9ϕC13, T10ϕC13 and T11ϕC13 were also generated in *B. thailandensis* strains T9, T10 and T11 after infection by the phage, ϕC13. In addition, the T19ϕC32 lysogen was also detected when the *B. thailandensis* T19 strain was infected with the phage ϕC32.

To confirm the phage-producing ability of these 6 lysogens, they were induced with 0.5 µg/ml of mitomycin C to release phages. The lysates were tested for lytic reactions on their *B. thailandensis* recipient strains. Results showed that these *B. thailandensis* lysogen lysates were able to form plaques, which resembled those seen in their donor lysates. Thus, we proposed that these lysogens came from phage infection, since their recipient strains were unable to produce phage after treatment with mitomycin C (data not shown).

Electron Microscopic Morphology and Nucleic Acid Type of the Purified Phages

Phage morphology was observed by TEM using negative staining with 2% PTA. The electron micrographs showed the morphology of the three phages, ϕC2, ϕC13 and ϕC32 (Fig. 1), which belonged to two types of Ackermann classification, A1 and C1, based on the morphology of phage-heads and tails[16]. Each of the 3 samples contained only one phage morphotype and the nucleic acid of all phages was DNA. Electron micrographs of phages ϕC2 and ϕC13 showed that their phage-heads were an isometric hexagonal shape about 55 to 71 nm in diameter, and they had a tail with a contractile sheath about 105 to 136 nm long (Fig. 1A and 1B). Based on the phage's morphology and nucleic acid type, ϕC2 and ϕC13 can be classified as Ackermann type A1 in the *Myoviridae* family. In contrast, the phage, ϕC32, belonged to Ackermann type C1 in the *Podoviridae* family, due to its isometric hexagonal head and short tail (Fig. 1C). The

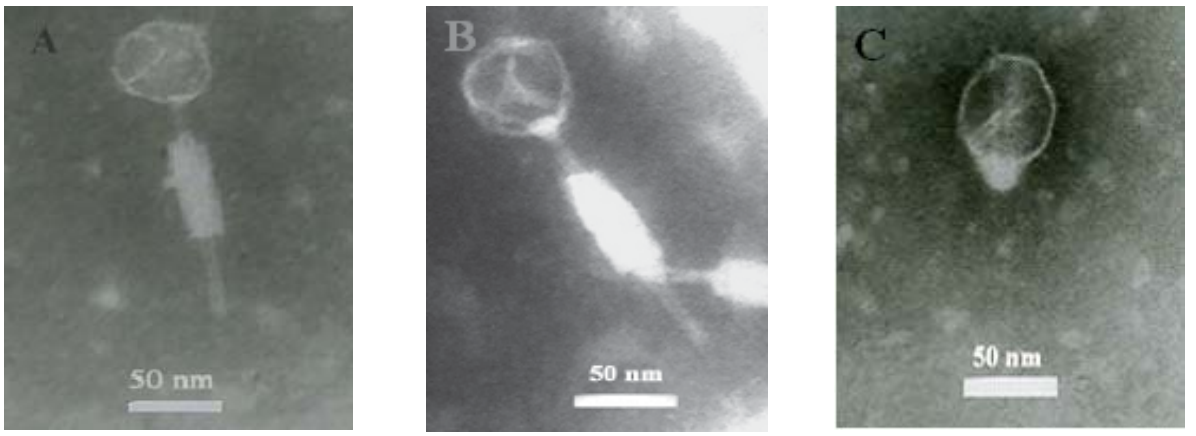

Fig 1. Transmission electron micrographs of phages induced from phage-donor strains negatively stained with 2% PTA. (A) ϕC2; (B) ϕC13; (C) ϕC32.

isometric hexagonal head of the ϕC32 phage was approximately 51 to 59 nm in diameter and the non-contractile knob, which was proposed to be its tail, was about 15 to 17 nm long.

Protein Profile of Whole Cell Lysates from Lysogens, Phage-Donor and Phage-Recipient Strains

To compare the protein profiles of whole cell lysates from lysogens, and phage-donor and phage-recipient

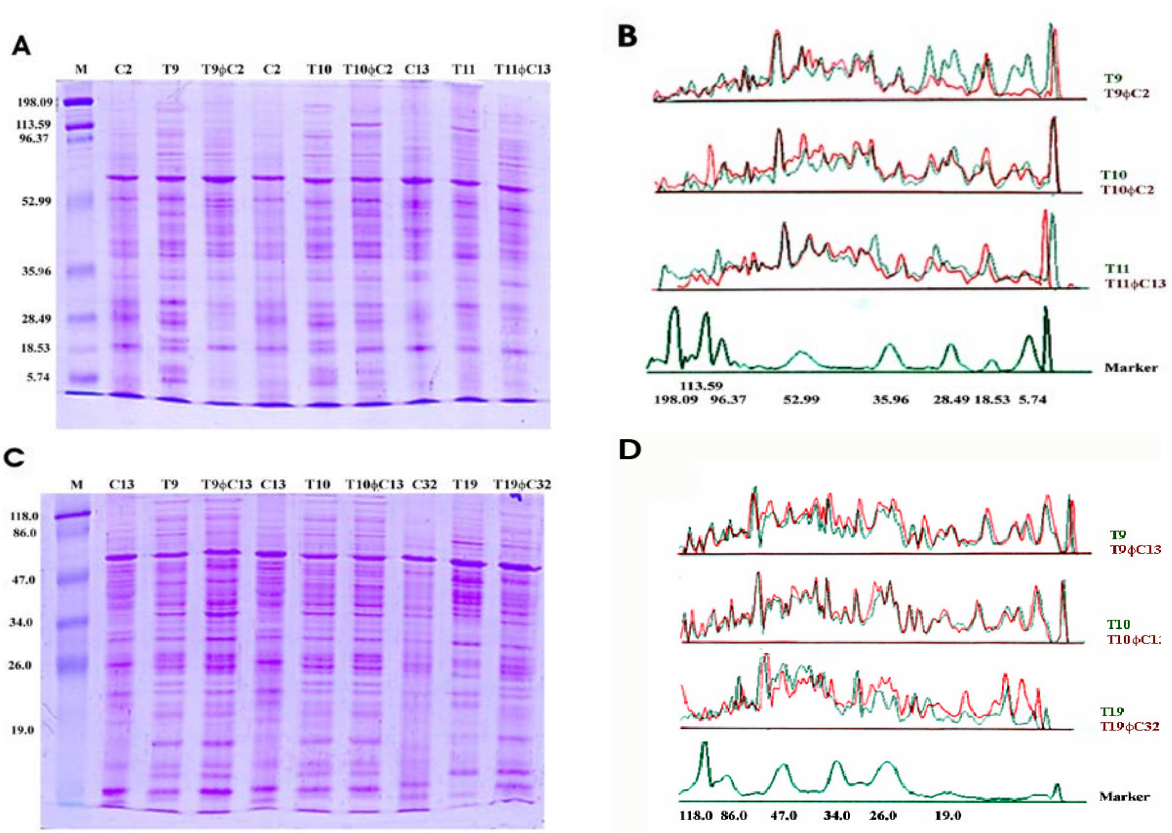

Fig 2. Protein profiles from whole cell lysates of phage-donors C2, C13, and C32, phage-recipients T9, T10, T11 and T19 and their lysogen strains on 15% gel SDS-PAGE. The bacterial cells were washed, resuspended in TE buffer and homogenized by sonication. (A and C) Clarified lysates (5 µg per well) were analyzed and stained with Coomassie blue R 250. Molecular weight markers are shown in kDa; (B and D) Densitograms showing protein profiles of phage-recipients and lysogens. Green line: phage-recipients; red line: lysogens.

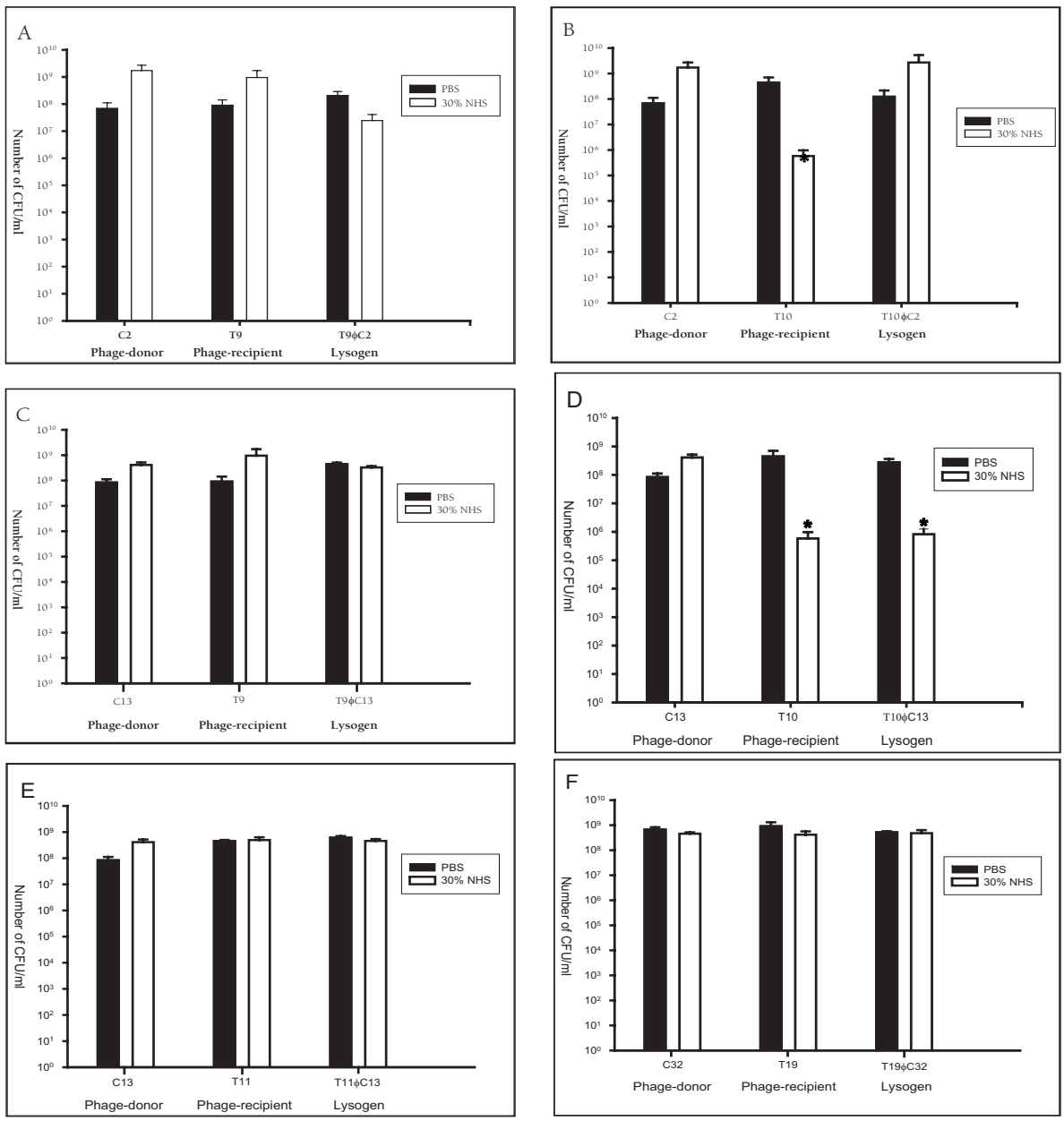

Fig 3. Serum bactericidal assay on a microtitre plate. Approximately 10^7 bacteria were incubated in 30% NHS for 2 h at 37°C and colony counts were expressed as the number of CFU/ml. The numerical values are the mean of three separate experiments ± standard deviation (bar). * significant difference for more than 2 log. (A) Phage-donor C2, phage-recipient T9 and lysogen T9ϕC2; (B) Phage-donor C2, phage-recipient T10 and lysogen T10ϕC2. (C) Phage-donor C13, phage-recipient T9 and lysogen T9ϕC13; (D) Phage-donor C13, phage-recipient T10 and lysogen T10ϕC13. (E) Phage-donor C13, phage-recipient T11 and lysogen T11ϕC13; (F) Phage-donor C32, phage-recipient T19 and lysogen T19ϕC32.

strains, whole cell lysates were prepared by sonication and the clarified lysates were separated on 15% SDS-polyacrylamide gel and stained with Coomassie blue R 250. These protein bands were also analyzed by the Image J program for easy comparison. The protein profiles between lysogens and recipients were compared. The positions of bands that had a difference in intensity in at least 2 of the three experiments were recorded.

Interestingly, two of six pairs of protein profiles between a lysogen and its recipient showed distinctively different profiles. In the first pair, the protein bands of

the T9ϕC2 lysogen showed a substantial difference from the T9 recipient (Fig. 2A). Protein bands with molecular weights ranging from 5.7 to 18.5 kDa and 28.5 to 30.0 kDa of the T9ϕC2 lysogen were decreased in intensity two to three fold when compared with the phage-recipient strain, T9, and they mimicked the phage-donor strain, C2 (Fig 2A and 2B). Similar results were obtained in three separately repeated experiments.

In the second pair, a protein band of the T10ϕC2 lysogen and its recipient strain, T10, with a molecular weight of 20 kDa, showed a slight decrease in intensity when compared with the phage-recipient strain, T10 (Fig. 2A). This observation was found in several repeat experiments.

Noticeably, in some experiments, the bands of the T9ϕC2 and T10ϕC2 lysogens, with a molecular weight of approximately 51 and 114 kDa, respectively, also showed a distinct increase in intensity when compared with these lysogen's recipients (Fig. 2A and 2B). Unfortunately, this difference did not occur again in repeat experiments.

For other lysogens and their recipient pairs, there was no clear difference in protein profiles on 15% SDS-PAGE. Their profiles did not meet our criteria of a difference being detected in at least 2 of the three experiments (Fig. 2).

Resistance to Serum

The ability of lysogens to multiply in PBS containing 30% NHS for 2 h was compared with their recipient and donor strains, and the total numbers of colony forming units per milliliter (CFU/ml) were determined. As a control for these studies, bacteria were inoculated in PBS without NHS.

Figure 3 shows that growth of the phage-donor strains in 30% NHS were not significantly different in comparison with the control. These results indicated that all of the phage-donor strains, C2, C13 and C32, as well as their phage-recipients, T9, T11 and T19 were resistant to 30% NHS. Only one of the phage-recipient strains, T10, was susceptible to death from 30% NHS, with a significant decrease in the colony numbers of more than 2 log when compared with the control. Surprisingly, while T10 was susceptible, the T10ϕC2 lysogen and its phage-donor strain, C2 (Fig. 3B), were resistant to 30% NHS and able to multiply in it. On the other hand, the T10ϕC13 lysogen was significantly susceptible when compared with the control, and it resembled the T10 phage-recipient strain in susceptibility despite the incorporation of the phage genome (Fig. 3D).

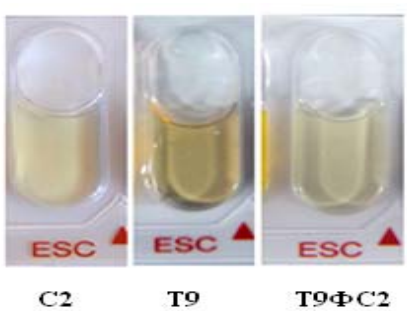

Fig 4. Esculin hydrolysis by *B. pseudomallei* phage-donor C2, *B. thailandensis* phage-recipient T9 and its lysogen T9ϕC2. In contrast to its phage-recipient (T9), the T9ϕC2 lysogen failed to hydrolyze esculin, similar to the phage-donor strain C2.

Biochemical Tests

API 20NE strips (Biomérieux, Marcy-l' Etoile, France) were used to characterize lysogens, phage-donor and phage-recipient strains by 20 different biochemical tests. Although the biochemical profiles did not distinguish *B. thailandensis* from *B. pseudomallei*, they detected a distinct difference in one parameter, i.e. *B. thailandensis* assimilated L-arabinose, whereas *B. pseudomallei* did not. In addition, these two species varied in esculin hydrolysis, which was normally positive in only 5% of bacterial strains tested (data from API 20NE database leaflet). A biochemical test by API 20NE showed that the T9ϕC2 lysogen, like its phage-donor strain, C2, was unable to hydrolyze esculin (Fig. 4).

Discussion

Bacteriophages can be categorized into two groups, temperate phage and virulent phage, based on their lifecycle. Temperate phages often encode properties that alter the host bacterium following the establishment of lysogeny. Prophages are responsible for much of the laterally transferred DNA in bacteria and they play a major role in the evolution of bacterial pathogens by providing new virulence determinants[17,18]. On the other hand, prophages may encode enzyme(s) or regulatory factor(s) that alter the bacterial host to reveal virulence. Living together in the same environment, closely related species of the bacteria are able to exchange or transfer some genetic materials to their neighbors through phages. Several reports demonstrated that phage can play a role in the pathogenesis of the host cells[19-25]. Despite the high percentage of lysogenicity in *B. pseudomallei,* and the ability of their temperate phages to lyse other related species, a phage of *B. pseudomallei* which plays a role in pathogenesis has not been reported. Whether the infected phages alter the phenotypes of the host bacteria has yet to be investigated.

In this study, we lysogenized *B. thailandensis* with

phages induced from *B. pseudomallei* and compared the phenotypes of these lysogens with their *B. thailandensis* recipient strains without phage infection. The induction of phages from 64 *B. pseudomallei* showed that 21 strains (32.8%) could produce phages which lyse the *B. thailandensis* strains included in our study. Lysogenization of *B. pseudomallei* detected in our study was lower that in the study of Manzeniuk et al. Ninety-two percent of their *B. pseudomallei* strains were lysogenic[9]. This was probably due to the fact that we detected phages from lysates by observing the lytic ability on our 22 isolates of *B. thailandensis*. It is possible that we missed many additional phages that cannot use these *B. thailandensis* isolates as a host. Owing to the limitation of phage producing strains, it was demonstrated that only three phages were able to lysogenize and convert 4 isolates of *B. thailandensis* to 6 lysogens. Phages induced from these lysogenic strains were similar in morphotype to those induced from their donor strains. These results indicated that the phage induced from *B. pseudomallei* infected *B. thailandensis* and resulted in lysogeny.

Although the existence of polylysogeny has been reported in *B. pseudomallei*[5], under the growth conditions used in this work, we found only one morphotype of phage in one strain of *B. pseudomallei*. It is worth noting that the phage-donor strains resisted infection from their own phage (data not shown), presumably due to the immunity or superinfection exclusion proteins encoded by the prophage they harbor.

Previously, bacteriophages that infect *Burkholderia* spp. fell into the A1 and A2 type in the *Myoviridae* family[16] and B1 type in the *Siphoviridae* family[10,11]. In this study, electron microscopic analysis of the lysates revealed that the induced phages, designated phage ϕC2 and ϕC13, belonged to the A1 morphotype of the family *Myoviridae*. Another phage, ϕC32, was classified as a C1 type, a member of the family *Podoviridae*. Since the phages of *Burkholderia* spp. reported previously have not fallen into the C1 type classification [10,11,16], the ϕC32 phage should be regarded as a new morphotype of phages isolated from *Burkholderia* spp.

To compare the protein profiles of lysogens and phage-recipient strains, the sonicated whole cell protein was separated on 15% SDS-polyacrylamide gels and stained with Coomassie blue. The results showed that SDS-PAGE profiles of T9ϕC2 and T10ϕC2 lysogens were undoubtedly different from their phage-recipient strains (Fig. 2A and 2B). The protein bands at molecular weights ranging from 5.7 to 18.5 kDa and 28.5 to 30 kDa of the T9ϕC2 lysogen and a protein band of the T10ϕC2 lysogen at a molecular weight of approximately 20 kDa, were decreased in intensity when compared with their phage-recipient strains (Fig. 2A and 2B). We also observed protein bands in lysates of T9ϕC2 and T10ϕC2 lysogens at molecular weights of approximately 51 and 114 kDa, respectively, which were different from their recipient strains (Fig. 2A and 2B). However, these differences could not be confirmed by repetitive experiments.

Protein profiles of the other 4 pairs were not clearly different and could not be detected in three repetitions (Fig. 2C and 2D). These variations in the protein bands may be due to a minor difference in the phase of growth within the bacterial population. The expression of some specific proteins can be different, or the efficiency of cell disruption at different phases of growth resulted in these unrepeatable detected bands. Moreover, these variations may also come from the heat generated during the sonication step that could denature some bacterial proteins.

Protein separation on SDS-PAGE has limitations because it could not separate proteins with a different isoelectric point but similar molecular mass. A comparison of protein profiles can be improved on two-dimensional electrophoresis, which can distinguish between other pairs.

Serum bactericidal assay showed that the T10ϕC2 lysogen was resistant to death from 30% serum, similar to the C2 donor strain, whereas the T10 recipient strain was susceptible. For disease-producing bacteria, their initial contact with the host defense mechanism upon penetration of the tissue barrier is, normal serum that plays a significant role in the protection against infection. Previous evidence indicated that *B. pseudomallei* is resistant to serum bactericidal activity, thus resistance to serum is probably a key virulence determinant of these bacteria[14,26,27].

The mechanism by which *B. pseudomallei* is resistant to lysis by human serum is unknown. Hence, factors may include interference with complement activation, effective opsonization, or complement-mediated lysis. A possible mechanism was explained by Egan and Gordon, who investigated the interaction of *B. pseudomallei* with complement, in the presence and absence of a specific antibody, to determine potential mechanisms of serum resistance. The results showed that rapid activation and consumption of complement by *B. pseudomallei,* in the absence of a specific antibody, occurred predominantly via an alternative pathway[26]. They also demonstrated the deposition and degradation of C3 and C5b-9 deposition on the surface of the bacteria. However, C5b-9 was eluted by 1 M NaCl, which suggested that it was ionically bound to the bacterial surface. Since the nature of the binding site for the membrane attack complex (MAC) on the outer cell membrane of bacteria has strong hydrophobic interaction, resistance to complement mediated lysis may result from the deposition of MAC in a nonmicrobicidal location[26]. In addition, DeShazer et

al. showed that the type II O-PS moiety of LPS was not present in the serum-sensitive Tn5-OT182 mutants of *B. pseudomallei* strains[27]. A representative serum-sensitive mutant was less virulent than the parental strain in hamsters, infant diabetic rats and guinea pigs. The results indicated that the type II O-PS moiety of LPS was required for serum resistance. The exact mechanism of type II O-PS-mediated serum resistance is currently unknown, but DeShazer and his colleagues proposed that the type II O-PS may be directly responsible for preventing the integration of the MAC into the bacterial outer membrane[27]. Since the LPS O antigen was required for the initial infection of the *B. pseudomallei* phage[10], our ϕC2 phage may attach to *B. thailandensis* T10 by using the LPS O antigen as a receptor and change the conformation of the LPS O antigen, thus achievling serum resistance. However, further examination is required.

The physiology of lysogens, their phage-donors and phage-recipients were determined by a biochemical test using the API 20NE kit. Results demonstrated that the T9ϕC2 lysogen could not hydrolyze esculin, similar to the C2 donor strain, while T9 could. Indeed, esculin is a β-D-glucose-6, 7-dihydroxycoumarin ($C_{15}H_{16}O_9 \bullet xH_2O$), the molecule can be hydrolyzed by β-glucosidase at the beta linkage to yield two end products, glucose and esculetin (6,7-dihydroxycoumarin). It may be possibly that during ϕC2 phage infection, the function of a β-glucosidase of the phage-recipient strain, T9, was interrupted or affected, thus, the T9ϕC2 lysogen was unable to hydrolyze esculin. Further investigations are required to understand this observation.

Three phenotypic characters, protein profiles, serum bactericidal assay and biochemical tests of lysogenized *B. thailandensis* were determined. The T10ϕC2 lysogen showed significant resistance to serum, while T10 was susceptible. The biochemical profiles showed that the phage-recipient strain, T9, could hydrolyze esculin, while, the T9ϕC2 lysogen could not. The phenotypic changes of two recipient-lysogenic pairs could also be detected by SDS-PAGE protein profiles. It may be interesting to see whether these protein profile changes were related to serum resistance or the biochemical profile.

On the other hand, DNA sequencing of the ϕC2 phage and analyzing its sequence will open a molecular view on the phage-bacterial relationship and generate information on gene(s) for regulatory factor(s) or enzyme(s) concerning esculin hydrolysis or serum resistance. The study of the bacteriophage of the *B. pseudomallei* on a larger scale, as well as the study of mechanisms and genetic factors related to melioidosis, may help in understanding the epidemiology of this pathogen in an endemic area, especially if *B. thailandensis* causes disease after lysogenic conversion by a phage from a *B. pseudomallei* pathogen.

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