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Phenotype and Virulence Assessment of a *Burkholderia pseudomallei* Soil Isolate from Malaysia

(Penilaian Fenotip dan Kevirulenan Pencilan Tanah *Burkholderia pseudomallei* dari Malaysia)

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ABSTRACT

Burkholderia pseudomallei*, a Gram-negative soil saprophyte, is the causative agent of life-threatening melioidosis.* B. pseudomallei *from soil and water remains a common source of human and animal infection* via *skin abrasions, ingestion or inhalation. Despite the reported sero-prevalence in healthy individuals among Malaysian rice farmers, there are limited reports on* B. pseudomallei *isolated from water or soil around the country. In this study, we characterized a* B. pseudomallei *soil isolate and compared it to local clinical isolates. 16s rRNA sequencing was adopted to confirm the identity of the soil isolate, NC20.* B. pseudomallei *NC20 colony morphology,* in vitro *growth rate and antibiotic sensitivity were examined and compared to two* B. pseudomallei *clinical isolates, UM6 and D286. Virulence properties such as biofilm formation and infection in a nematode host were also examined. The soil isolate NC20 exhibited distinguishable features of* B. pseudomallei*, comparable growth rate and similar antibiotic resistance profile to UM6 and D286. Additionally, NC20 is a medium-level biofilm producer with levels similar to D286, where the amount of biofilm produced was much less relative to UM6. Interestingly, NC20 exhibited weaker killing of the* Caenorhabditis elegans *infection model relative to the clinical isolates. The comparison between soil-derived and clinical isolates of* B. pseudomallei *demonstrated that both soil and clinical isolates shared certain phenotypic properties but the soil isolate was somewhat less virulent than the clinical isolates used in this study*.

Keywords: B. pseudomallei*; biofilm; soil isolate; virulence*

ABSTRAK

Burkholderia pseudomallei*, bakteria Gram negatif saprofit tanah, ialah agen penyebab penyakit melioidosis.* B. pseudomallei *daripada tanah dan air ialah sumber utama jangkitan manusia dan haiwan melalui lecetan kulit, pengingesan atau pernafasan. Walaupun terdapat laporan kelaziman kehadiran antibodi pada individu sihat di kalangan pesawah padi tempatan, laporan pemencilan* B. pseudomallei *daripada tanah atau perairan di seluruh negara masih terhad. Di dalam kajian ini, kami telah mencirikan satu pencilan* B. pseudomallei *dari tanah dan seterusnya membandingkannya dengan pencilan klinikal* B. pseudomallei *tempatan*. *Penjujukan 16s rRNA telah digunakan bagi menentusahkan identiti pencilan tanah tersebut yang dinamakan NC20*. *Analisis morfologi koloni, lengkungan pertumbuhan bakteria* in vitro *serta analisis sensitiviti terhadap antibiotik dilakukan ke atas* B. pseudomallei *NC20 dan dibandingkan dengan pencilan klinikal* B. pseudomallei*, UM6 dan D286. Ciri kevirulenan seperti pembentukan biofilem dan jangkitan terhadap perumah nematod juga telah dijalankan. Pencilan tanah NC20 menunjukkan ciri yang mirip* B. pseudomallei*, menunjukkan kadar pertumbuhan bakteria yang serupa serta profil kerintangan antibiotik yang sama dengan UM6 dan D286. Tambahan pula, NC20 adalah pembentuk biofilem sederhana setara dengan D286 dengan kadar pembentukan biofilem adalah lebih rendah berbanding UM6. Menariknya, NC20 menunjukkan kadar pembunuhan model jangkitan* Caenorhabditis elegans *yang lemah apabila dibandingkan dengan pencilan klinikal. Perbandingan antara pencilan tanah dan klinikal* B. pseudomallei *menunjukkan kedua-dua pencilan tanah dan klinikal berkongsi ciri fenotip tertentu, tetapi bakteria pencilan tanah mempunyai tahap kevirulenan yang lebih rendah berbanding pencilan klinikal yang digunakan dalam kajian ini.*

Kata kunci: B. pseudomallei*; biofilem; kevirulenan; pencilan tanah*

Introduction

Burkholderia pseudomallei is a Gram-negative, soil dwelling saprophyte which is endemic in northern Australia and Southeast Asia, including Malaysia (Puthucheary et al. 1992; White 2003; Wiersinga et al. 2012). It is the causative agent of melioidosis, a potentially fatal bacterial infection in humans and animals. Clinical manifestations of melioidosis vary widely among patients; the disease could present as asymptomatic, manifest as localized soft-tissue infection or acute septicemia with pneumonia. The growing risk of *B. pseudomallei* acquired antibiotic resistance has been well documented (Schweizer 2012) whereby resistance to antimicrobials include third generation cephalosporins, quinolones, and aminoglycosides (Puthucheary & Sam 2012). Resistance to ceftazidime, the first-line antibiotic therapy for *B. pseudomallei* infections, has led to patient fatalities if ceftazidime administration is not complemented with a different antibiotic (Khosravi et al. 2014). Furthermore, *B. pseudomallei* is capable of persisting in a dormant state, and as a result of this, the bacteria is not completely cleared from a patient and this could result in recurrent infection (Wiersinga et al. 2018). In Thailand, a relapse rate of 15% per year has been reported despite prolonged antibiotic therapy (Limmathurotsakul et al. 2016).

B. pseudomallei infection generally occurs *via* skin abrasions, ingestion or inhalation. Melioidosis cases have been frequently reported in farmers and agriculture workers proposing that their frequent contact with soil and standing water is responsible for the high number of cases reported. The presence of *B. pseudomallei* in soil and water reservoirs throughout Malaysia is highly likely due to the climatic condition of the country that is favorable for its growth in the environment. In Kedah, a state in Malaysia with the highest rice production, the reported incidence rate is 16.35 melioidosis cases per 100,000 population (Hassan et al. 2010). Pahang, the largest state in Peninsular Malaysia where agriculture is the main economic activity, recorded incidences of culture-confirmed adult melioidosis of 6.1 per 100,000 population per year from 2000-2003 (How et al. 2005). Further to this, ten confirmed melioidosis cases were reported after a rescue operation at a waterfall in Pahang resulted in 70% of the first responders succumbing to melioidosis (Sapian et al. 2012). Although the probability of contracting *B. pseudomallei* from the environment is considerably high, there are limited reports describing soil or water isolates from Malaysia. Hence, a comparative analysis between environmental and clinical *B. pseudomallei* isolates is quite urgent. Insights into bacterial pathogenicity and antibiotic sensitivity of environmental isolates may provide useful information for administration of appropriate antibiotics whilst knowledge of potential differences in colony morphology may improve diagnosis of *B. pseudomallei* infection using the gold standard bacterial culture.

Here we report on the characterisation of a Malaysian *B. pseudomallei* soil-derived isolate, NC20, in comparison to two local clinical isolates, UM6 (Chin et al. 2015) and D286 (Lee et al. 2007). We provide a comparison between colony morphology, antibiotic sensitivity and virulence properties such as biofilm formation as well as infection in a nematode infection model host.

Materials and Methods

Bacterial isolates and *Caenorhabditis elegans* strain

The *B. pseudomallei* strains used in this study are listed in Table 1. All *B. pseudomallei* strains were kept at -80 °C and routinely cultured on Ashdown selective agar when required. All experiments involving *B. pseudomallei* were performed in a BSL2+ level laboratory. *C. elegans glp-4* mutant strain was obtained from the Caenorhabditis Genetics Center (CGC) and propagated on Nematode Growth Medium (NGM) supplemented with *Escherichia coli* strain OP50 as the food source.

TABLE 1. Description of *B. pseudomallei* strains used in this study

B. pseudomallei strain	Description	Reference
NC20	Soil isolate from a football field in Kuala Kangsar, Perak	This study
UM6	Clinical isolate from blood specimen	Koh et al. 2013
D286	Clinical isolate from Kuala Lumpur Hospital, Malaysia	Lee et al. 2007
R15	Clinical isolate from Institute for Medical Research, Kuala Lumpur	Lee et al. 2007
H10	Clinical isolate from Raub General Hospital, Pahang	Lee et al. 2007

16S rRNA AMPLIFICATION AND SEQUENCE ANALYSIS

B. pseudomallei NC20 genomic DNA was extracted using the MasterPure™ DNA Purification Kit (Epicentre, Wisconsin, USA) and used as template for amplification of the 16S rRNA gene. Each PCR reaction (25 µL) contained 1U GoTaq polymerase (Promega Corporation, Madison, USA), 1X Green GoTaq R Flexi Buffer, 0.2 mM dNTP mix, 4.0 mM $MgCl_2$ and 0.35 µM of each primer (primer U1: 5' ACGCGTCGACAGAGTTTGATCCTGGCT 3'; primer U2: 5' CGCGGATCCGCTACCTTGTTACGACTT 3'). Amplification was carried out in the S1000™ Thermal Cycler (Bio-Rad, California, USA) with the following parameters: 96 °C for 3 min (1 cycle), 96 °C for 15 s (30 cycles), 60 °C for 90 s (30 cycles), 72 °C for 2 min (30 cycles) and 72 °C for 5 min (1 cycle). Amplicons were purified with the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) before Sanger sequencing. The obtained sequence was matched against the NCBI non-redundant database using BLASTn and aligned to the 16S rRNA sequence of the *B. pseudomallei* reference strain (K96243) with CLUSTALW (https://www.genome.jp/tools-bin/clustalw).

IN VITRO GROWTH ANALYSIS

B. pseudomallei NC20, UM6, and D286 were cultured overnight in Luria-Bertani (LB) broth at 37 °C. Overnight cultures were adjusted to OD_{595}= 0.15 in LB. The OD-adjusted culture (500 µL) was diluted 100× in 50 mL fresh LB broth and incubated at 37 °C with shaking at 250 rpm. Throughout the incubation period, an aliquot of the culture was taken at specific intervals and diluted appropriately where 10 µL of the diluted culture was spotted on Ashdown agar followed by incubation for 48 h. The mean doubling time for individual strains was calculated using the equation $g= t \log_{10}2/(\log_{10} N_t - \log_{10} N_0)$ where N_t is the number of cells at a later time point in the exponential phase, N_0 is the number of cells at an early time point in the exponential phase and t indicates the time interval (in minutes) between N_0 and N_t (Maier 2009).

DISC DIFFUSION TEST

The disc diffusion test was carried out with commercial antibiotic discs (Pronadisa, Madrid, Spain) for evaluating susceptibility to sulfamethoxazole (100 µg), ceftazidime (30 µg), meropenem (10 µg) and trimethoprim (10 µg) or self-prepared paper discs infused with tetracycline (30 µg), doxycycline (30 µg), kanamycin (30 µg), chloramphenicol (30 µg), gentamicin (100 µg), streptomycin (100 µg), ampicillin (30 µg), and carbenicillin (100 µg). Briefly, an overnight culture of individual *B. pseudomallei* strains was adjusted to OD_{595} = 0.5 (10^8 cfu/mL) and spread across Mueller-Hinton (MH) agar to form a uniform lawn. The discs were placed on the lawn followed by incubation at 37 °C for 24 h. The formation of a clear inhibition zone around the discs indicated the susceptibility of *B. pseudomallei* to the specific antibiotic(s). Specific breakpoints were determined on the interpretative criteria defined for *B. pseudomallei* (Thibault et al. 2004) or the standard interpretative criteria of non-Enterobacteriaceae by the Clinical and Laboratory Standard Institute (CLSI) (CLSI 2014).

BIOFILM FORMATION ASSAY

The biofilm assay was performed as previously described (Eng & Nathan 2015). Briefly, the overnight culture of each *B. pseudomallei* strain was diluted with Brain Heart Infusion (BHI) broth to OD_{595}= 1.0. The standardized culture (200 µL) of each *B. pseudomallei* strain was dispensed as inoculums, in triplicates, into a 96-well plate and incubated at 37 °C for 48 h. Uninoculated BHI broth was included as the negative control. Thereafter, the wells were washed twice with 1× Phosphate Buffer Saline (PBS) to remove non-adherent bacteria, followed by fixing with 200 µL 99% (v/v) methanol for 15 min. Upon air drying of the samples at room temperature, the wells were stained with 200 µL 2% crystal violet for 5 min. Excess stain was removed by washing three times with water and the wells were air-dried. Crystal violet stain was released from bacterial stains by solubilizing with 200 µL 95% (v/v) ethanol and the released stain was measured at 570 nm using a microplate reader (Sunrise, Tecan, Switzerland). Statistical analysis was performed using unpaired, two-tailed Student's t-test.

C. elegans KILLING ASSAY

A conditional sterile *C. elegans* mutant, *glp-4*, was used in this assay to avoid the presence of progeny in addition to the initial population of infected worms. In brief, worms were synchronized and grown to adult stage (Wong et al. 2016). Nematode eggs harvested from a pool of gravid worms were allowed to grow on NGM plates at 25 °C for 60 h. In parallel, *B. pseudomallei* infection plates were prepared accordingly. Each *B. pseudomallei* isolate was grown overnight in 3 mL BHI broth with 4 mg/mL gentamycin at 37 °C. Ten µL of an overnight culture was spread on a 3.5 cm NGM assay plate followed by incubation at 37 °C for 24 h and subsequently at room temperature for 1 to 24 h. Forty sterile worms were transferred to each infection plate and plates were incubated at 25 °C. Nematodes were scored as dead when they failed to respond to touch. Assay plates containing the uracil auxotroph *E. coli* strain OP50, the typical laboratory food for *C. elegans* maintenance, served as the negative control for infection.

Results

The soil *B. pseudomallei* isolate NC20, was isolated from a football field in Kuala Kangsar, Perak, Malaysia, and kindly donated by Prof. Dr. Abdul Karim Russ Hassan (UniKL, Malaysia). The identity of NC20 and clonal purity was first confirmed by 16S ribosomal RNA (rRNA) gene sequencing. Amplification of the 16S rRNA target region produced an amplicon of 1299 bp. Basic Local Alignment Search Tool (BLAST) analysis showed a perfect match (100% nucleotide identity) between the *B. pseudomallei* NC20 16S rRNA gene fragment and a list of *B. pseudomallei* strains, including the reference *B. pseudomallei* K96243 strain (GenBank accession CP009538.1) (data not shown). This confirmed NC20 as a contaminant-free, pure isolate of *B. pseudomallei*.

Soil isolates are exposed to a different environment compared to *B. pseudomallei* isolated from clinical samples. Hence, it is likely that colonies from both sources would exhibit some phenotypic differences as colony morphology is known to be highly variable in *B. pseudomallei*. In this study, *B. pseudomallei* NC20 colony morphology was examined with reference to two *B. pseudomallei* clinical isolates, strains D286 (Lee et al. 2007) and UM6 (Chin et al. 2015). The *B. pseudomallei* strains (NC20, D286 and UM6) were grown on agar for three days at 37 °C. As shown in Figure 1, NC20 shares extensive features with *B. pseudomallei* D286. Both exhibit a central rough surface with radiating wrinkles. In contrast, NC20 and UM6 were quite dissimilar from each other. While both NC20 and UM6 had a rough surface and irregular circumference, UM6 was more desiccated, thicker and a darker purple in colour. Nonetheless, we conclude that there is no significant difference in colony morphology between the soil and clinical isolates in this study.

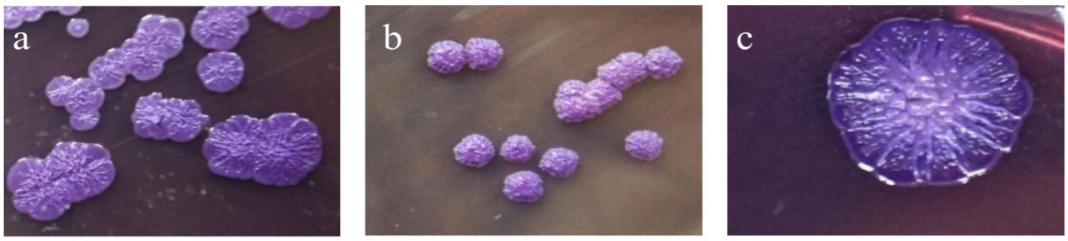

FIGURE 1. Colony morphology of *B. pseudomallei* strain (a) NC20, (b) UM6 and (c) D286 on Ashdown agar after 72 h incubation at 37 °C

To examine if soil and clinical isolates grow at different rates when cultured *in vitro*, a growth curve analysis was performed on *B. pseudomallei* NC20, D286 and UM6 in LB broth. Colony forming units (CFUs) per mL culture were enumerated over an incubation period of 24 h. As seen in Figure 2, no distinguishable difference in growth rate was observed between *B. pseudomallei* NC20, D286, and UM6. Similarly, there was no significant difference in doubling time between all three strains with average doubling times for *B. pseudomallei* NC20, UM6 and D286 of 56.47, 62.75 and 66.36 min, respectively.

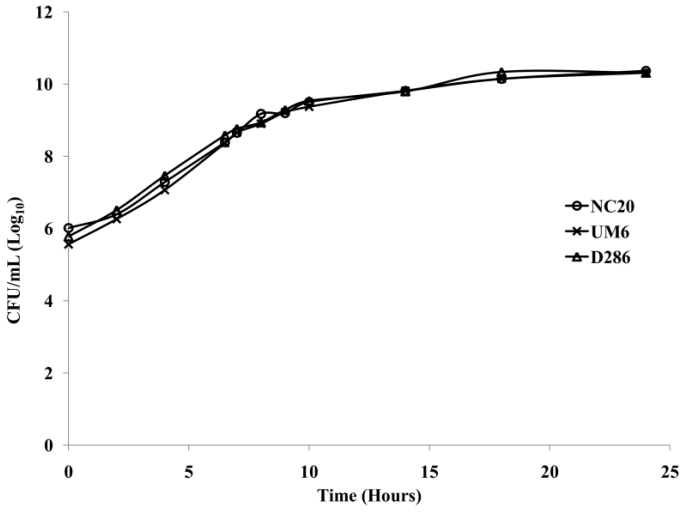

FIGURE 2. Colony forming units (CFUs) per mL culture of *B. pseudomallei* NC20, UM6 and D286 in LB

B. pseudomallei UM6 is a high biofilm producer (Chin et al. 2015) whereas the biofilm-forming abilities of *B. pseudomallei* NC20 and D286 are currently not known. Biofilm is a sessile bacterial community encapsulated in a protective exopolysaccharide (EPS) matrix. This protective layer confers resistance to antibiotics and is strongly associated with immune evasion leading to chronic and persistent bacterial infection (Leid 2009; Rodríguez-Martínez & Pascual 2006). Here, biofilm-forming ability of these three *B. pseudomallei* strains were compared. Absorbance of bacteria treated with crystal violet at the optical density of 570 nm which is indicative of biofilm formation, was measured at 48 h post incubation (Figure 3). As expected, *B. pseudomallei* UM6 produced biofilm at levels which were significantly higher than both NC20 and D286 (Student's t-test, $p < 0.001$). As there was no significant difference in biofilm production between NC20 and D286, this suggests that the biofilm-producing ability is likely to be strain-specific and is independent of the origin of the isolate. Indeed, it was previously reported that both high and low biofilm-producing strains are present among *B. pseudomallei* isolated from blood and pus (Koh et al. 2013).

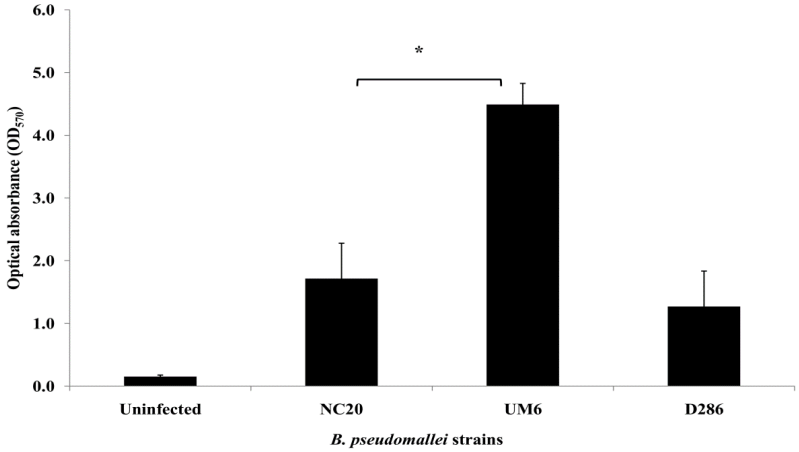

FIGURE 3. Biofilm formation of *B. pseudomallei* NC20, UM6 and D286 at 37 °C. The asterisk denotes the p-value calculated from *t*-tests ($p < 0.001$)

Biofilm formation has been identified as one of the attributes of antibiotic resistance in bacteria where the protective matrix acts as a diffusion barrier to antibiotics. Cells which aggregate in a biofilm layer can be 1000 times more resistant to antimicrobial agents than their planktonic counterparts (Gilbert et al. 1997). In view of the correlation between biofilm formation and antibiotic resistance, we examined differential antibiotic susceptibility of the soil (NC20) and clinical isolates (UM6 and D286). In response to different environmental exposure, soil and clinical isolates may vary in terms of their antibiotic susceptibility profile. Soil isolates may exhibit resistance to a broader range of antimicrobials as a result of having to deflect antimicrobials produced by neighboring environmental bacteria. On the other hand, *B. pseudomallei* clinical isolates are expected to have higher acquired resistance toward clinical antibiotics. *B. pseudomallei* NC20, UM6, and D286 were grown in the presence of thirteen antibiotics (Table 2) and bacterial sensitivity or resistance was observed. As summarized in Table 2, the soil isolate NC20 exhibited a similar antibiotic resistance profile as the two clinical isolates for every antibiotic tested. These results are concordant with a previous study which reported that the antibiotic resistance

profiles of *B. pseudomallei* and *Burkholderia mallei* were independent of the origin of isolates (Thibault et al. 2004). Of the antibiotics tested, *B. pseudomallei* NC20, UM6, and D286 were sensitive to sulfamethoxazole (100 µg), ceftazidime (30 µg), meropenem (10 µg), tetracycline (30 µg) and doxycycline (30 µg). Of note, ceftazidime, meropenem, trimethoprim and doxycycline are antibiotics that are currently used to treat melioidosis patients. As both the soil and clinical *B. pseudomallei* isolates showed similar sensitivity toward these antibiotics, the current standard antibiotics regimen should be as effective for treating *B. pseudomallei* infections that originate from the soil.

TABLE 2. Antibiotic susceptibility profile of *B. pseudomallei* NC20, UM6 and D286 towards a panel of twelve antibiotics

Antibiotic	Amount (µg)	*B. pseudomallei* strains		
		NC20	UM6	D286
Sulfamethoxazole	100	S	S	S
Ceftazidime	30	S	S	S
Meropenem	10	S	S	S
Trimethoprim	10	R	R	R
Tetracycline	30	S	S	S
Doxycycline	30	S	S	S
Kanamycin	30	R	R	R
Chloramphenicol	30	R	R	R
Gentamicin	100	R	R	R
Streptomycin	100	R	R	R
Ampicillin	30	R	R	R
Carbenicillin	100	R	R	R

R: Resistant; S: Sensitive

There is a fundamental assumption that high bacterial growth rates contribute to increased virulence whilst the presence of high amounts of biofilm is also implicated as a virulence determinant. Thus far, we have demonstrated that the soil isolate NC20 grows at a rate similar to the clinical isolates tested whilst biofilm formation by NC20 was lower than the known biofilm producer UM6 but similar to D286. To examine if a *B. pseudomallei* soil isolate has virulence potential similar to clinical isolates, we performed a nematode survival assay of *C. elegans* infected with *B. pseudomallei* NC20, UM6, and D286. To reinforce the comparison, we included two other *B. pseudomallei* clinical strains in this assay, R15 (highly virulent in the worm and mouse models) and H10 (low virulence in the worm and mouse infection models) (Lee et al. 2007). Age-matched *glp-4* worms were exposed to individual strains and worm survival was calculated as mean-time-to-death (TD_{mean}) where a shorter TD_{mean} indicates superior bacterial pathogenicity. As shown in the nematode killing curve (Figure 4), the high biofilm producer, UM6, was the most virulent strain with a TD_{mean} of 18.09 ± 0.167 h, followed by R15 (TD_{mean} of 25.73 ± 0.848 h), D286 (TD_{mean} of 28.09 ± 0.534 h), NC20 (TD_{mean} of 48.47 ± 0.815 h), and H10 (TD_{mean} of 50.73 ± 0.788 h). Based on these results, *B. pseudomallei* clinical strains generally exhibited a higher killing capacity relative to NC20, albeit a similar killing rate was observed for NC20 and H10.

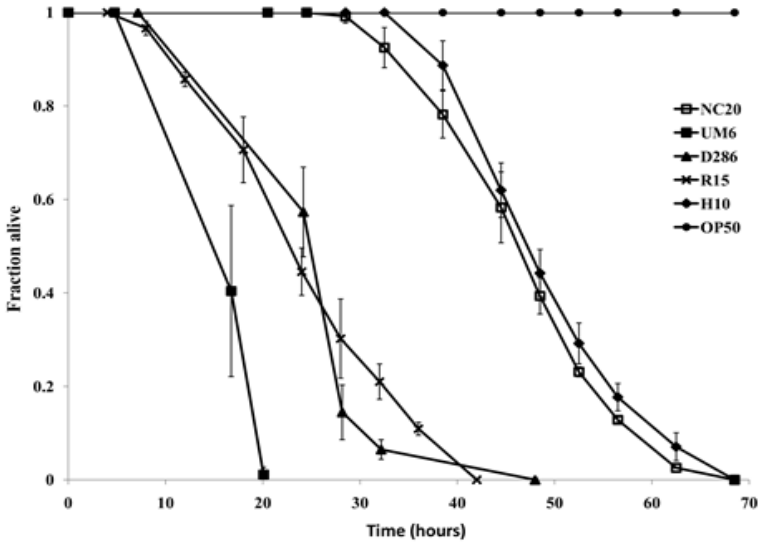

FIGURE 4. Nematode killing curve of glp-4 *C. elegans* worms infected by *B. pseudomallei* NC20, UM6, D286, R15, and H10. In general, NC20 infected worms died significantly slower than those infected by clinical isolates. Error bars represent mean value ± SD

DISCUSSION

Several studies have reported variations within colony morphology in *B. pseudomallei*. We previously reported four different morphologies for *B. pseudomallei* animal-derived and clinical isolates, including D286, grown on Ashdown media and Luria-Bertani agar (Lee et al. 2007). At least seven different morphotypes of *B. pseudomallei* with distinguishable features have been previously noted (Chantratita et al. 2007; Chen et al. 2009). These morphotypes could reversibly switch to other isogenic types as adaptation strategies to specific growth conditions. Interestingly, in those studies, phenotypic alterations correlated well with bacterial virulence. More than one study reported the association between colony morphology and bacterial pathogenicity. For example, a wrinkled and dry morphotype was more lethal in mice with significant organ damage compared to its isogenic mucoid and semi-dry counterpart (Chen et al. 2009). Likewise, mice infected with mucoid, soil-derived strains demonstrated an apparent prolonged survival where a secondary infection was required to kill the mice (Chen et al. 2014). Soil-derived isogenic *B. pseudomallei* strains have been reported to exhibit mucoid and smooth colony morphologies, however, we did not observe this morphotype for *B. pseudomallei* NC20. Instead, this particular soil isolate exhibited features that resemble the Type I morphotype, the major *B. pseudomallei* morphotype found among isolates from Thailand (Chantratita et al. 2007). Meanwhile, *B. pseudomallei* D286 exhibited similar morphology as NC20 whilst UM6 showed features of a Type II morphotype based on the classification of Chantratita et al. (2007).

No differences were obeserved in growth rate and antibiotc resistance between the soil and clinical isolates. Nevertheless, NC20 and UM6 demonstrated an average biofilm production whilst UM6, as expected, produced significant amounts of biofilm. These observations were consistent with the previous report in which the Type II morphotype stands out as a strong biofilm producer (Chantratita et al. 2007). We also showed that UM6 was the most highly virulent strain tested in this study. The high virulence potential is likely ascribed to the high biofilm production by UM6 as the association between the biofilm production and bacterial virulence has been well documented (Begun et al. 2007; Chin et al. 2015). However, we believe that biofilm is not the sole factor contributing to the differential virulence, as D286 demonstated a more superior killing ability than NC20, despite both having similar capacity for biofilm production.

The *C. elegans* infection data suggests that, in general, clinical strains are more virulent than environmental

strains. However, this suggestion requires further validation by testing a larger cohort of representative *B. pseudomallei* environmental strains. To this end, a previous study reported a disparity in terms of correlation between the origin of isolates and *Burkholderia* pathogenicity in the *C. elegans* model. Cardona et al. (2005) demonstrated that while 78% of *Burkholderia cepacia* complex environmental strains were pathogenic to *C. elegans*, only 52% of the clinical isolates exhibited pathogenic traits. The observed difference in virulence may also be attributed to differences at the genome level. Preliminary sequence analysis of *B. pseudomallei* H10, which is of similar low virulence as NC20, has indicated the absence of a number of hypothetical genes that could encode for unique virulence factors (Ghazali et al. - unpublished). Bartpho et al. (2012) proposed that the absence of certain genomic islands in environmental *B. pseudomallei* strains could explain the lower virulence potential of the soil isolates. Furthermore, genome sequence descriptions of globally acquired *B. pseudomallei* strains have reiterated the initial findings from Holden et al. (2004) that the genomic content of this bacterial pathogen is highly plastic due to frequent recombination events.

A limitation of this study is the description of only one environmental isolate with the absence of a clinical isolate from the corresponding location in Perak to enable a more extensive comparative phenotypic analysis. However, the isolation of *B. pseudomallei* from environmental sources is problematic and generally results in limited positive samples. A more extensive surveillance program for melioidosis in Malaysia could identify the epidemiological distribution of the bacteria, melioidosis hotspots and associated environmental risk factors. Nonetheless, this present study has proposed that it is likely that *B. pseudomallei* isolates from different sources share a common repertoire of genes whose regulation determines survival and fitness in different environments. Comparative genome and transcriptome analysis between NC20 and a virulent clinical isolate such as UM6 may shed more light.

Conclusion

This study provides information on the phenotype and virulence potential of a Malaysian *B. pseudomallei* environmental (soil) isolate NC20. The soil isolate is highly similar to the clinical isolates examined in this study in terms of colony morphology, growth curve and antibiotic sensitivity profile. Nevertheless, the congruent phenotype among the strains did not translate to a similar virulence profile. We noted that D286 shared the same colony morphology and biofilm production with NC20 but was significantly more virulent than NC20. Collectively, the soil isolate was less virulent toward *C. elegans* compared to the clinical isolates. However, any suggestion of a link between the source of isolate and virulence is only speculative at this juncture and requires the evaluation of more environmental isolates. It is possible that the weaker virulence of NC20 is strain-dependent and is independent of the origin of the isolate. Nevertheless, the phenotypic relatedness between clinical and environmental isolates suggests that *B. pseudomallei* infections are indeed acquired from the environment.

Acknowledgements

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