Research Article

Physicochemical Determinants of Distribution of Sequence Types (ST) of *Burkholderia pseudomallei* from Small Ruminant Farms in Peninsular Malaysia

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Abstract | Physicochemical properties of the soil or water environment within which microorganisms dwell have been shown to influence the presence of *Burkholderia pseudomallei*. A total of 78 isolates, of which 56 are from soil and 22 from water obtained from livestock farm environments, were molecularly characterized by multilocus sequence typing (MLST) and analyzed against the environmental physicochemical properties from 33 livestock farms in four selected states of Negeri Sembilan, Pahang, Perak, and Selangor in Peninsular Malaysia. Multinomial logistic regression analysis found a significant association between soil water content and ST (sequence type) 84 (OR = 0.833, 95% CI: 0.708 to 0.980; p=0.027) when compared to ST51. This showed a unit increase in soil water contents was associated with a 1.2 times increase in the odds of recovering ST51 compared with the odds of recovering ST84. Also, a statistically significant protective association was recorded between water pH and ST84 (OR=0.401, 95% CI 0.195-0.828; p = 0.013) when compared to ST51. This showed that for a unit increase in water pH, there was a 2.5 times increase in the odds of recovering ST51 compared with the odds of recovering ST84. These findings suggested that variation in the occurrence of various *B. pseudomallei* STs is associated with variations in the environmental (soil and water) physicochemical factors. Physicochemical properties such as soil water content and water pH might have influenced the distribution of certain genotypes of *B. pseudomallei* in the endemic areas. This information could be useful for planning control programs tailored toward environmental interventions to reduce contamination in non-endemic areas.

Keywords | *Burkholderia pseudomallei*; Sequence type; Physicochemical properties; Environment; Soil; Water.

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INTRODUCTION

Properties or characteristics of the environment where soil or water micro-organisms reside have been shown to influence strain types (Ngamsang et al., 2015; Islam et al., 2020; Pandit et al., 2020). The saprophytic β-proteobacteria *B. pseudomallei* causes melioidosis, a potentially fatal infectious disease that affects both humans and animals. The organism is known to be endemic in certain parts of Southeast Asia and Australia and within these areas are pockets, where the organism is thought to occur at higher frequencies (Baker et al., 2015; Pongmala et al., 2022; Birnie et al., 2022). The biogeographical distribution of *B. pseudomallei* can be distinct and is reported to be associated

with the physicochemical attributes of the. In endemic countries, the organism may not be evenly distributed throughout the landscape (Limmathurotsakul et al., 2010; Jayasinghearachchi et al., 2021). This uneven distribution of *B. pseudomallei* is considered to be due to physical and chemical factors in the environment that have been found to affect microbial populations in the soil (Ngamsang et al., 2015; Chewapreecha et al., 2022). In Malaysia, melioidosis is an emerging infectious disease among livestock and small ruminants are known to be most susceptible. A previous study by Musa et al. (2015) reported that certain farm practices such as lime treatment which improves soil alkalinity are associated with a reduced likelihood of isolation of *B. pseudomallei* from the environmental soil samples. In addition, the distribution of seropositivity against melioidosis among livestock was shown to be significantly different between states (Musa et al, 2012) in Peninsular Malaysia which may be speculated to be due to the differences in the soil properties.

The effects of environmental factors on the survival and persistence of *B. pseudomallei* under experimental conditions have been well documented (Tong et al., 1996; Inglis and Sagripanti, 2006; Larsen et al., 2013; Paksanont et al., 2018). The level of environmental factors such as levels of copper, iron, and zinc, pH, carbon-nitrogen ratio (C: N), exchangeable calcium (EC), soil moisture, texture, and organic matter contents have been reported to correlate with the presence of *B. pseudomallei* (Ngamsang et al., 2015; Baker et al., 2015). Acidic and soft water with low salinity but high iron contents were associated with the occurrence of this organism (Draper et al., 2010). The natural properties of the local environment that may affect the variation in the distribution of the strains of this organism have not been investigated.

In this study, we investigated different molecular genotypes (sequence types) of *B. pseudomallei* isolated from soil and water concerning the physical and biochemical characteristics of these sources. It is believed that the knowledge of the relationship between environmental physicochemical properties (variables/factors) and *B. pseudomallei* sequence types (STs) can help in understanding the distribution of the organism and options for control in a given area based on the physicochemical property of sources.

MATERIALS AND METHODS

Sources of isolates and resuscitation procedure

Isolates of *B. pseudomallei* obtained from soil and water samples from livestock farms from an earlier study by Musa et al. (2015) were used in this study. The isolates were preserved at -20°C in a broth medium comprised of 20% glycerol-brain heart infusion (BHI) sourced from Oxoid (Basingstoke, United Kingdom). The isolates were preserved at the Veterinary Bacteriology Laboratory, Faculty of Veterinary Medicine Universiti Putra Malaysia (UPM). A total of fifty-six (56) isolates from soil and 22 from water samples from livestock (small ruminant) farms' environments were obtained. The study design and bacteriological analyses performed have been described by Musa et al. (2015).

Genomic DNA extraction and PCR confirmation of isolates

The genomic DNA of *B. pseudomallei* isolates was extracted using Qiagen DNeasy® (Qiagen, Germany) genomic DNA extraction kit for bacterial cultures according to the manufacturer's instructions. The DNA template obtained was diluted with sterile PCR-grade DNase-free water (Qiagen, Germany) to a final concentration of 50ng/µL. A spectrophotometer (BIO-RAD, California) was used to test the purity of the extracted DNA by quantification through measurement of the absorbance at 260 nm and 280 nm using the cut-off point for A_{260}/A_{280} ratio was 1.8. Template DNA having an A_{260}/A_{280} ratio ≥ 1.8 was considered pure

Polymerase chain reaction (PCR) amplification of the 550bp gene fragment was achieved through the use of *B. pseudomallei* specific 16S rRNA region according to the work of Brook et al. (1997). A PCR mixture of 50 µl final volume that comprised 25 µL of Top Taq Master Mix® (Qiagen, Germany),5 µL of 1 X Concentrate Coral Load, 0.5 µM of Forward Primer; 0.5 µM of reverse Primer, 0.25 µg DNA template and 13 µL sterile DNase-free water. The PCR mixture was run in a thermal cycler (BioRad, USA) for 30 cycles of 1 min at 94°C initial denaturation, annealing at 54°C for 30 sec, and extension at 72°C for 2 min, with a final extension step at 72°C for 10 min (Brook et al., 1997). Gel electrophoresis of the PCR products was assessed for amplification using 1.5% agarose gel in 1.5 times TBE and run for one hour at 80 m. volts and Gel Viewer (BioRad, USA) was to view evidence of amplification.

Multilocus sequence typing (MLST)

The multilocus sequence typing (MLST) procedure was carried out per the MLST website, http://bpseudomallei.mlst.net. The sequences of seven housekeeping genes of *B. pseudomallei* that include *ace*, *gltB*, *gmhD*, *lepA*, *lipA*, *nark*, and *ndh* were obtained from the MLST website (http://bpseudomallei.mlst.net). For the amplification of each of these genes, loci was performed as described earlier by Sadiq et al. (2018). Briefly, a PCR mixture of 50µl final volume that comprised 25 µL of Top Taq Master Mix® (Qiagen, Germany), 5 µL of 1 X Concentrate CoralLoad, 0.5 µM of Forward Primer; 0.5 µM of reverse Primer, 0.25 µg DNA template and 13 µL sterile DNase-free water

was carried out according to Sadiq *et al.* (2018). Sterile PCR-grade water was used in place of the DNA template to serve as a negative control. The amplification was carried out according to the PCR protocol described on the MLST website (http://bpseudomallei.mlst.net). The PCR mixture was run in a thermal cycler (BioRad, USA) for 4 min at 95°C initial denaturation, followed by 30 cycles of denaturation at 95°C, for 30 sec, annealing at 62°C for 30 sec, and extension at 72 °C for 1 min, with a final extension step at 72 °C for 10 min and cooled to 4°C (http://bpseudomallei.mlst.net). Gel electrophoresis of the PCR products was assessed for amplification using 1.5% agarose gel in 1.5 times TBE and ran for one h at 80volts and Gel Viewer (BioRad, USA) was to view evidence of amplification.

All procedures were conducted in a biosafety level two (BSL II) facility at an accredited laboratory, the Faculty of Veterinary Medicine, UPM. In addition, personal protective equipment (PPE) was used throughout the procedures. All PCR reagents, mixture preparations, and product purifications were carried out in a sterile environment of the BSL II facility. MEGAquick-spin™ (iNtRON Biotechnology, Korea) DNA purification kits were used to purify PCR products according to the manufacturer's instructions and the Sanger sequencing method (Sanger et al., 1980) was carried out using the same primers used for the initial amplification. The housekeeping gene sequences obtained were trimmed to the appropriate size for each locus using Biological Sequence Alignment and Editing (BioEdit) version 7.19 (Tom Hall, Ibis Biosciences® Carlsbad CA, USA). The sequences of each housekeeping gene were queried on the MLST database to determine the allele number sequence type (ST) and new alleles and STs were submitted to the MLST database curator for verification and assignment of allele numbers and STs as described by Sadiq et al. (2018).

Source of environmental physicochemical properties data

Data for environmental variables were obtained for 33 livestock farms located in the states of Pahang, Perak, Selangor, and Negeri Sembilan of Peninsular Malaysia. The data on the environmental physicochemical properties have been described in a previous study by Musa et al. (2015). These include data on soil water and organic matter contents, soil texture, soil pH, soil carbon, nitrogen and sulfur contents, and soil trace element contents that included manganese, copper, iron, and zinc. Others include the contents of basic cations in the soil such as potassium, sodium, calcium, and magnesium as well as the cation exchange capacity of the soil. For the water samples, the pH, and dissolved oxygen content were measured as described in the previous study by Musa et al. (2018). Briefly, an analysis of the soil texture was carried out based on the method described by Miller and Miller (1987) while soil organic matter contents analysis was based on the method described by Salehi et al. (2011). Soil water content analysis was based on the method of Forster (1995) the soil trace elements analysis, soil cation exchange capacity, and soil basic cations contents were determined using the method of Sarojam (2009). A soil pH meter (Radiometer, Copenhagen Denmark) was used to measure soil pH while soil carbon, nitrogen, and sulfur contents were analyzed by a CNS analyzer (TruMac CNS analyzer, Leco, USA).

Data analysis

The data generated were entered into Microsoft Office Excel for Windows. The statistical analyses were performed using SPSS (Version 22.0; SPSS Inc., Chicago, IL). Initial screening of the individual environmental factors variables was done using univariate regression analysis using an alpha (α) level of 0.05. Variables that were found to be significant at α level 0.05 and those with P value ≤ 0.25 were selected. The selected variables were subjected to a pairwise Pearson correlation coefficient (r) to check for multicollinearity. Variables were considered strongly correlated if $r \geq 0.80$ to exclude collinear variables. The association between each of the environmental factor variables with the dependent/outcome variables was assessed using a multinomial logistic regression model. The dependent/ outcome variables were the four 'ST categories': ST46, ST51, ST84, and other STs (in which ST164, ST1130, ST1131, and ST1339 were merged). Using a forward stepwise approach in which explanatory variables that were significant with $P < 0.05$ were selected, a multiple logistic regression model was developed in which explanatory variables that were significant with $P < 0.05$ were selected and entered into the final model. Variables were removed if the P value was > 0.25 but did not significantly alter the point estimates of the variables that remained in the model.

The dataset used for this study can be obtained from the corresponding author upon request.

RESULTS

Univariate analysis of soil and water physicochemical properties

The univariate analysis of the variance of soil and water variables is presented in Table 1. Only eight out of the 22 environmental (soil and water) variables namely soil water content (%), soil pH, soil Fe (mg/kg), Zn (mg/kg), sand percentage, silt percentage, soil sulphur, and water pH were significantly associated with ST category of the *B. pseudomallei* isolates. Pairwise Pearson correlation analysis of these eight variables did not reveal any strong correlation, hence there is no multicollinearity among them. These en

Table 1: Univariate analysis of soil and water physicochemical properties

Env. Variable	Mean square	F	p-value
Soil water content (%)	5.808	8.730	0.004*
Soil organic matter content	0.523	0.195	0.660
Soil pH	2.348	4.997	0.028*
Soil CEC	7320.906	3.177	0.079
Fe (mg/kg)	142877.883	8.781	0.004*
Cu (mg/kg)	0.242	0.964	0.339
Zn (mg/kg)	1.257	5.562	0.021*
Mn (mg/kg)	0.220	0.649	0.423
Na (mg/kg)	0.032	0.580	0.449
Mg (mg/kg)	0.018	0.984	0.324
Ca (mg/kg)	0.001	0.000	0.989
K (mg/kg)	182.392	1.615	0.208
Percentage sand	3439.327	5.959	0.017*
Percentage clay	0.966	0.073	0.788
Percentage silt	3521.122	7.790	0.007*
Soil carbon	0.161	0.195	0.660
Soil nitrogen	0.003	0.494	0.484
Soil sulphur	0.001	5.549	0.021*
Water pH	3.625	4.780	0.032*
Water oxygen content	0.523	0.195	0.660
Water dissolved oxygen	0.001	0.001	0.976
Water optical density	0.009	0.510	0.478
Water chemical O_2 demand	184321.991	0.338	0.563

Key: Iron (Fe), Copper (Cu), Manganese (Mn), Zinc (Zn), Sodium (Na), Magnesium (Mg), Calcium (Ca), Potassium (K) and Cation exchange capacity (CEC). *Significant at α= 0.05

Table 2: Multinomial logistic regression analysis of soil properties based on the STs of *Burkholderia pseudomallei*

Variable	Sequence type	β	SE	p-value	OR	95% CI for OR	
	ST51 (n=35)	Ref			1.00		
Soil water content	ST46 (n=15)	0.14	0.11	0.18	1.15	0.94	1.42
	ST84 (n=22)	-0.18	0.08	0.03*	0.83[a]	0.71	0.98
	Other STs (n=6)	-0.25	0.15	0.112	0.78	0.58	1.06
Zn (mg/kg)	ST51 (n=35)	Ref			1.00		
	ST46 (n=15)	-2.62	1.50	0.08	0.07	0.01	1.38
	ST84 (n=22)	0.66	0.68	0.33	1.94	0.51	7.40
	Other STs (n=6)	1.29	0.77	0.09	3.63	0.80	16.38

Key: Zinc (Zn), Odds ratio (OR), Confidence interval (CI) and Standard error (SE)
[a]The odds of isolation of *B. pseudomallei* ST84 is 0.83 (95% CI 0.71-0.98) times compared with ST51. That is the odds of isolation of *B. pseudomallei* ST84 decreased with increases in soil water content when compared with ST51
*Significant at α= 0.05

Table 3: Multinomial logistic regression analysis of water properties based on the STs of *Burkholderia pseudomallei*

Variable	Sequence type	β	SE	p-value	OR	95% CI for OR	
Water pH	ST51 (n=35)	Ref			1.00		
	ST46 (n=15)	-0.27	0.35	0.44	0.77	0.39	1.58

ST84 (n=22)	-0.91	0.37	0.01*	0.40[a]	0.19	0.83
Other STs (n=6)	-1.19	0.70	0.09	0.30	0.08	1.20

Key: Ref (Reference), Odds ratio (OR), Confidence interval (CI) and Standard error (SE)
[a]The odds of isolation of *B. pseudomallei* ST84 is 0.40 (95% CI 0.19-0.83) times compared with ST51. That is the odds of isolation of *B. pseudomallei* ST84 decreased with increases in water pH when compared with ST51
*Significant at α= 0.05

-vironmental variables that are significant at P≤ 0.25 were then selected for inclusion in multinomial logistic regression analysis. Soil Fe, sand percentage, and silt percentage were excluded the fact that their mean squares were too large.

Logistic regression analysis of soil and water properties with the STs of Burkholderia pseudomallei isolates

Multinomial logistic regression was performed to investigate whether independent variables (soil physicochemical properties) predict the dependent (outcome) variable (ST category of *B. pseudomallei* isolates), which has four categories, ST46, ST84 and other STs with ST51 as the base or comparison category. Although six soil and two water variables assessed by univariate analysis were statistically significant (P < 0.05), only five of the soil variables were found fit to be included in the multinomial logistic regression model using the forward stepwise variable selection approach.

Table 2 shows the result of the multinomial logistic regression analysis of soil variables based on the STs of *B. pseudomallei* isolates. A statistically significant protective association was detected between soil water content and ST84 (OR = 0.83, 95% CI 0.71-0.98; p= 0.03) when compared to ST51. This shows that the odds of recovery of *B. pseudomallei* ST51 are 1.2 times higher with an increase in soil water content when compared with the odds of recovery of ST84. There was no statistically significant association between the water content of the soil with any of the ST46 and other STs categories. There was no statistically significant association between the zinc content of the soil with any of the ST categories.

Multinomial logistic regression analysis between the water pH and ST categories of *B. pseudomallei* demonstrated a statistically significant protective association between water pH and ST84 (OR=0.401, 95% CI, 0.195-0.828; p= 0.01) when compared with ST51. This shows that the odds of recovering *B. pseudomallei* isolates with ST84 decreased with unit increases in the pH of water. This means that for unit increases in soil pH, there was a 2.5 times increase in the odds of recovering ST51 compared with ST84. There was however no statistically significant association between water pH and ST46 or other STs when compared to ST51 (Table 3).

DISCUSSION

Certain environmental physicochemical properties have been hypothesized to affect the environmental persistence of the soil-dwelling saprophytic bacterium, *B. pseudomallei* in endemic regions (Inglis and Sagripanti, 2006; Ngamsang et al., 2015; Baker et al., 2015, Birnie et al., 2022). Zueter et al. (2018) did not show evidence of an association between any of *B. pseudomallei* STs and the clinical presentation of melioidosis. Their study revealed that there was no statistically significant association between bacterial genotype clusters and clinical outcomes. Teh et al. (2021) revealed an increased diversity of *B. pseudomallei* in Peninsular Malaysia where confined evolution gives rise to the emergence of new STs, suggesting that environmental factors (soil and water) might have played a role in the evolutionary variability in *B. pseudomallei*. The *in-vitro* investigations of various factors in the environment that support the survival of *B. pseudomallei* in soil were reported to include the pH, osmotic (salt), soil texture, and chemical stress (Inglis and Sagripanti, 2006; Wang-ngarm et al., 2014; Duangurai et al., 2018). The presence of *B. pseudomallei* may be predicted by the direct measurement of the soil physicochemical properties from which the organisms reside (Palasatien et al., 2008; Birnie et al., 2022). The present study is unique because some physicochemical factors that influence the presence of certain strains of *B. pseudomallei* among positive soil samples were identified. At present, published studies on the physicochemical properties of soil and water that influence the genotype of *B. pseudomallei* found in the environment are scanty. This is probably the first study that examines the relationship between environmental physicochemical properties (variables/factors) and the occurrence of *B. pseudomallei* multilocus sequence types (STs) distribution in environmental soil and water samples.

In this study, multinomial logistic regression revealed that soil water content had a significantly associated protective effect on the occurrence of ST84 compared to ST51. The odds of recovering *B. pseudomallei* ST84 decreased with increases in soil water content when compared with that of ST51. This in turn signifies that increased soil water content is a risk for the occurrence of ST51, where there were 1.2 times more chances of recovering ST51 with an increase in soil water content compared to ST84. As there are no available studies that have attempted to identify environmental factors influencing the distribution of the

B. pseudomallei genotype, we could not directly compare our findings with others from previous studies. However, among other studies that have attempted to identify physicochemical risk factors between soil that are positive for the organism compared to those that are negative, soil moisture (water) content was observed to be one of the common soil factors alongside high rainfall and surface water that were associated with the distribution of clinical melioidosis in the wet tropics (Palasatien et al., 2008). Palasatien et al. (2008) demonstrated significantly higher soil water content among *B. pseudomallei* positive soil samples compared with negative soil samples. Tong et al. (1996) have observed that death of the *B. pseudomallei* occurs within 70 days with a decrease in soil water content below 10% whereas a water content above 40% maintained the viability of the bacteria for up to 726 days. This also supported the previous studies that demonstrated an increase in the isolation of *B. pseudomallei* from soil samples with an increase in soil water content of ≥10% (Palasatien et al., 2008; Wang-ngarm et al., 2014; Goodrick et al., 2018). The availability of water in the soil water is essential for bacterial survival (Palasatien et al., 2008; Goodrick et al., 2018), which is also influenced by soil texture. Suebrasri et al. (2013) observed that soils with larger particles lower the soil's water retention capacities whereas soil with smaller particles allows them to hold more water. For that reason, other soil properties may also influence the level of soil moisture content which may, in turn, affect the distribution of the *B. pseudomallei* STs.

The significant protective association between soil water content and the isolation of ST84 as compared with ST51 implies that it would be less likely to isolate *B. pseudomallei* ST84 in soils with high water content. The results highlighted from this research may require more investigation and in-depth investigation. However, it could mean that ST84 is better at resisting low soil moisture levels compared with ST51. For the precise mechanisms for these phenomena, some strains within the same species have been known to be more resilient to environmental stressors and inhibitors as reported for clinical *B. pseudomallei* K96243 (Korbsrisate et al., 2005).

Even though this study found that the association between the STs and zinc was not statistically significant, zinc was included in the final model because of its biological plausibility. Zinc has been reported to play roles in some metabolic processes that involved activities of metalloenzymes, expression of exoproteases and proteins essential in the *B. pseudomallei* survival and pathogenicity (Percheron et al., 1995; Liew et al., 2013; Burtnick and Brett, 2013). Multinomial logistic regression demonstrated a near-significant protective association between ST46 when compared to other STs. Soils with relatively high zinc concentrations as compared to ST51. The effect of levels of zinc in the soil on the distribution of *B. pseudomallei* has not been consistently similar in the available literature. For example, Baker et al. (2015) reported that low zinc concentrations favored the survival of *B. pseudomallei*, whereas in a study carried out in rice paddy from Northern Laos, Manivanh et al. (2013) reported that Zn concentrations do not affect the presence of *B. pseudomallei*. This study, however, suggested that Zn level may influence the strains or type of *B. pseudomallei* ST that is present in the soil hence; more investigation is recommended on the effects of Zn on the distribution of *B. pseudomallei* strains in the soil.

This study demonstrated that lower water pH may reduce the likelihood of the occurrence of ST84 when compared with ST51. Even though no study can be found that compared *B. pseudomallei* genotype preferences to environmental factors such as water pH, strain preferences of pH have been demonstrated for other microorganisms such as *Helicobacter pylori* and *Staphylococcus aureus* that are specialized to tolerate extreme acidic pH and salinity conditions respectively (Robinson et al., 2011). The variation in pH tolerance in *B. pseudomallei* could be attributed to the fact that these environmental saprophytic organisms are usually able to tolerate wide ranges of pH (Tong et al., 1996; Wang-ngarm et al., 2014; Duangurai et al., 2018). In a previous study that investigated water properties preferred by the organism in China, *B. pseudomallei* isolates were shown to survive in acidic water (water pH 3) for up to seven days (Tong et al., 1996). Another study by Finkelstein et al. (2000) reported the recovery of *B. pseudomallei* from water sources ranging in pH levels from 2 to 9. When compared to other related organisms such as *B. cepacia* and *Pseudomonas aeruginosa*, *B. pseudomallei* has the highest adaptability to acidic environments (Dejsirilert et al., 1991; Duangurai et al., 2018). Similar to the other findings in this work, it can be deduced that an in-depth study that is beyond the scope of the present study is required to explain the variations observed in this study.

CONCLUSION

Some environmental physicochemical factors have a statistically significant association with the occurrence of soil and water-dwelling bacterium *B. pseudomallei*. The water content of the soil was found to have had a significant statistical association with the occurrence of ST84 of the *B. pseudomallei* while water pH levels were also found to be significantly associated with the recovery of the ST84 strains of *B. pseudomallei*. The odds of recovering the ST51 strain of *B. pseudomallei* were higher in both soil with higher water content and higher pH compared to the odds of recovering strain ST84. These suggest differences in the preference of various *B. pseudomallei* STs for different eco-

logical niches. The information on the phylogenetic variability and the relationship between the *B. pseudomallei* genotype and environmental factors can be used in the planning and evaluation of melioidosis control measures tailored toward environmental interventions by altering factors that reduce contamination in a non-endemic area. A more elaborate study needs to be done to elucidate the effects of environmental physicochemical factors on the occurrence of *B. pseudomallei* genotypes (STs) and the mechanisms involved in these variabilities are hereby recommended.

CONFLICT OF INTEREST

The authors have no competing interests to declare that are relevant to the content of this article.

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NOVELTY STATEMENT

Environmental physicochemical factors that included soil water content and pH levels were found to have a statistically significant association with the occurrence of certain sequence types of *B. pseudomallei* in Peninsular Malaysia. The sequence types affected by these factors were the ST84 and ST51 of the bacteria, *B. pseudomallei*.

AUTHORS CONTRIBUTIONS

MAS, LH, SAA, and ZZ designed the study. LH, ZZ, and SAA supervised the research. MAS performed the experimental work, while HIM and MMA carried out the isolation of the bacterial cultures. MAS, HIM, and LH drafted, corrected, and reviewed the manuscript. All authors read and approved the final manuscript.

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DATA AVAILABILITY

The datasets utilized for this study are available from the corresponding author on request.

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