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Physicochemical Factors Affecting the Growth of *Burkholderia pseudomallei* in Soil Microcosm

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Abstract. *Burkholderia pseudomallei* causes melioidosis, the third most common cause of death from infectious diseases in northeast Thailand. Four physicochemical factors were set so that their values covered the range of the northeast, which is an endemic area. The soil pH was set at pH 4–10, soil salinity was 0.0–5.0% NaCl, total iron was 50–150 mg/kg soil, and carbon to nitrogen ratio (C/N) was 10:1 to 40:1. The experiments were carried out at 37°C, and soil moisture was maintained for 7 days. The number of viable bacterial cells was counted daily. Soil pH, salinity, Fe, and C/N ratio affected the bacterial growth. The bacterial colony was significantly ($P < 0.05$) reduced at soil pH > 8, soil salinity > 1% NaCl, and C/N ratio > 40:1. However, the growth of *B. pseudomallei* was enhanced by increasing the concentrations of iron significantly ($P < 0.05$). We propose using these findings to control *B. pseudomallei in situ*.

INTRODUCTION

Melioidosis is caused by *Burkholderia pseudomallei*, a saprophytic bacterium commonly found in soil and water in southeast Asia and northern Australia.[1,2] *B. pseudomallei* was classified by the US Centers for Disease Control (CDC) as a possible biological weapon.[3] Melioidosis patients were reported to be infected by inhalation[4–7] or percutaneous inoculation from contaminated muddy soil or stagnant water in endemic location, resulting in pneumonia[8–10] and sepsis, and up to 40% of treated patients died with this infection.[2,11,12] In Thailand, presence of *B. pseudomallei* in soil is the highest in the northeast.[13,14] Also, the incidence of melioidosis is the highest in this region.[15–17] The incidence of melioidosis was reported to be correlated with the monsoonal wet season in Khon Kaen, Thailand[18] and Townsville, Australia.[19–22] Importantly, a prospective cohort study in northeast Thailand during 1997–2006 revealed the increasing incidence of human melioidosis cases, and in fact, the disease is still a major public health issue in this region,[23] because it is the third most common cause of death from infectious diseases in the northeast of Thailand.[24]

Because of the correlation of incidence of melioidosis cases with season, the presence of *B. pseudomallei* is correlated with the physicochemical nature of its ecological habitat. A soil survey in the endemic area in Thailand revealed the correlation of the bacteria with low soil pH, moisture content > 10%, high chemical oxygen demand, and total nitrogen.[25] In addition, a soil survey in Australia using molecular techniques showed the association of *B. pseudomallei* with grasses, livestock animals, low soil pH, and different combinations of soil texture and color.[26]

The aim of this study is to investigate the effects of the physicochemical properties of soil on *B. pseudomallei* growth in soil microcosms. This study provides basic information on the factors that affect the survival/growth of the bacterium in soil, and the results may be applied to control the pathogen *in situ*.

MATERIALS AND METHODS

Preparation of *B. pseudomallei*. *B. pseudomallei* was isolated from site 39 (Ban Kai Na) in Nam Phong District, Khon Kaen Province, northeast Thailand; 100 g top soil at 30-cm depth were vigorously mixed with 100 mL distilled water and left for 30 minutes to allow sedimentation. Supernatant was transferred into the selective enrichment medium (threonine basal salt solution with 20 mg/L colistin [TBSS-20]). After incubating at 42°C at 200 rpm for 48 hours, 100 μL surface liquid were plated on the modified Ashdown's agar.[25] The medium was modified by changing Trypticase soy agar (TSA) at 15 g/L to tryptic soy broth (TSB) at 10 g/L and agar at 15 g/L and reducing gentamicin from 8 to 5 mg/L; it was incubated at 42°C for 4 days. *B. pseudomallei*-suspected colonies (wrinkled or smooth with purple-pink color) were counted and verified by triple sugar iron (TSI), augmentin/colistin susceptibility, assimilation of l-arabinose test (*B. pseudomallei* is negative), and latex agglutination.[27]

In each experiment, a single colony of *B. pseudomallei* isolated from the soil sample was grown in 5 mL Luria–Bertani (LB) broth overnight before being adjusted to an optical density (OD_{550}) of 0.1. It was subsequently diluted 1:100 in LB broth and incubated at 200 rpm at 37°C until OD_{550} of 0.8 (which is equal to 10^8 colony-forming units [CFUs]/mL). The numbers of bacterial cells were confirmed by colony counting on the modified Ashdown's agar plate before adding into soil microcosms.

Soil sample. Soil for preparing microcosm was collected from the *B. pseudomallei*-positive site (site 39; the same site as the *B. pseudomallei* collection). The soil sample was air-dried for 7 days and sieved to < 2 mm. The sieved soil was kept in a sealed plastic bag and stored at 4°C until use. Physicochemical characteristics of the soil sample, including soil texture, pH, electrical conductivity (EC), moisture content (MC), water holding capacity (WHC), organic matter (OM), organic carbon (OC), total Kjeldahl nitrogen (TKN), available phosphorus (P_{avai}), soil microbial biomass (SMB), iron, manganese, and aluminum, were determined. The methods of analysis and the results of soil properties are shown in Table 1.

Soil microcosm. Next, 100 g soil were placed into a 250-mL glass bottle. The treatment parameters in each batch were varied, and the initial moisture content was adjusted to 60% WHC using deionized water before autoclaving at 121°C at 15 psi for 15 minutes. The soil sterility was checked by suspending soil particles in a 2.5% wt/vol polyethylene glycol (PEG) and 0.1% wt/vol sodium deoxycholate (DOC) solution and plating on modified Ashdown's agar.[28] One milliliter *B. pseudomallei* inoculant (10^8 CFU/mL) was dropped over the soil in each bottle. To spread the bacterium evenly, a 100-μL pipette was used to drop the inoculums; two drops were

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TABLE 1
Summary of soil physicochemical properties

Soil parameters	Soil properties
Soil texture	Sandy loam
pH	5.60
EC	0.02 dS/m
MC	9.0%
WHC	22.5%
OM	0.29%
OC	0.17%
TKN	106.03 mg/kg
P_{avai}	6.5 mg/kg
SMB	86.7 μg g^{-1} soil
Total iron	50 mg/kg
Total manganese	31 mg/kg
Total aluminum	2.5 g/kg

dropped on each one-quarter of the soil surface, and two more drops were dropped around the center area. To maintain soil moisture during incubation between 50% and 60% WHC, the bottles were covered with aluminum foil and incubated at 37°C according to the method in the work by Chen and others.[29] The number of *B. pseudomallei* (CFUs) in the soil in each bottle was enumerated after 0, 1, 2, 3, 4, 5, 6, and 7 days using the drop plate method.[30] Triplicate soil microcosms were prepared for each treatment in two independent sets of experiment.

To maintain the soil moisture content during the experiments, each bottle was weighed after autoclaving, and the weight loss was randomly checked every 2 days. After 7 days of experiment, the final moisture content was within the expected range (51% of WHC).

Environmental factors affecting *B. pseudomallei* growth. Soil environmental factors used in this study are pH, salinity, iron, and C/N, and they cover the normal range of the physicochemical characters of soil in northeast Thailand. The details of microcosm settings are given below.

Soil pH. The pH of dry soil samples was adjusted by 6 M H_2SO_4 and 6 M NaOH to make the final pH near 4, 5, 6, 7, 8, 9, and 10 before autoclaving. The pH range used was determined by our preliminary results (pH 3, 5, 6, 9, and 12), because the bacterium could not be detected using culture method at pH 3, 9, and 12. Soil samples were left overnight, and their pH values were rechecked using a pH meter (EcoScan pH5; EUTECH, Singapore) at 1:1 (soil:water).[31] The soil pH in the soil microcosm was measured every day before extraction, and their pH values were not changed much throughout the experiment.

Soil salinity. Concentrated sodium chloride (NaCl) (AR grade; BDH Prolabo, VWR International, Lutterworth, UK) solution was added to dry soil samples to make various soil salinity values of 0%, 0.25%, 0.4%, 0.5%, 0.7%, 1.0%, 2.5%, and 5.0% NaCl (0.02, 1.19, 1.79, 1.89, 2.85, 4.15, 8.14, and 14.59 dS/m, respectively). The salinity used in this study was based on our preliminary experiments using 0%, 1.0%, 2.5%, 5.0%, 7.5%, and 10% salt in the soil microcosm. The results showed that more than 5% salt could limit the number of *B. pseudomallei*. The salinity of soil was determined using an electrical conductivity meter (CH-8603; Mettler Toledo, Switzerland) by extraction with water at a concentration of 1:5 (soil:water).[32]

Iron. Ferrous sulfate ($FeSO_4$) (AR grade, Ajax Chemicals PTY Limited, Auburn, NSW, Australia) was added to dry soil samples to achieve various iron concentrations of 50, 75, 100, 125, and 150 mg/kg in soil. The iron in the sample was measured by the acid digestion method and analyzed by an atomic absorption spectrophotometer (Perkin-Elmer Analyst 300).[33]

C/N ratio. The C/N ratio of soil microcosms was adjusted with urea (AR grade; BDH Prolabo, VWR International, Lutterworth, UK) as the nitrogen source to make the C/N ratios of 10, 15, 20, 25, 30, and 40. Soil organic carbon was measured by the method by Walkley and Black,[34] and total Kjeldahl nitrogen was determined by the Kjeldahl method.

Enumeration of *B. pseudomallei*. *B. pseudomallei* were determined from the soil microcosm by the PEG-DOC method[28]; 200 mL PEG-DOC solution containing 2.5% wt/vol PEG 6000 (AR grade; Merck KGaA, Darmstadt, Germany) and 0.1% wt/vol DOC (AR grade; Sigma-Aldrich, MO) were added to 100 g soil and vigorously shaken at 200 rpm for 2 hours. The mixture was allowed to stand for 5 minutes before the bacterial suspension was serially diluted in sterile normal saline solution (0.9% NaCl); 10 μL each dilution were plated in 10 replicates (100 μL in total) on modified Ashdown's agar using the drop plate method described in the works by Hoben and Somasegaran[35] and Herigstad and others.[30] The plates were incubated at 37°C and inspected visually daily for 4–7 days for colonies of *B. pseudomallei*.

Statistical analysis. All soil microcosm experiments were performed two times with three replicates of each treatment. The total sample size was six ($N = 6$). The results were analyzed by two-way analysis of variance (ANOVA) with Duncan's multiple range test for multiple comparisons among treatments using SPSS version 17 (SPSS).

RESULTS

Effects of soil pH on the growth of *B. pseudomallei*. *B. pseudomallei* in microcosms responded to soil pH differently. Bacteria could tolerate more at acidic pH than basic pH (Figure 1). It could survive and persist in soil at the pH range = 5–7. The growth rates and numbers of bacteria at all pH values were significantly different ($P < 0.05$) from the control (pH 5.0) within 7 days when analyzed by two-way

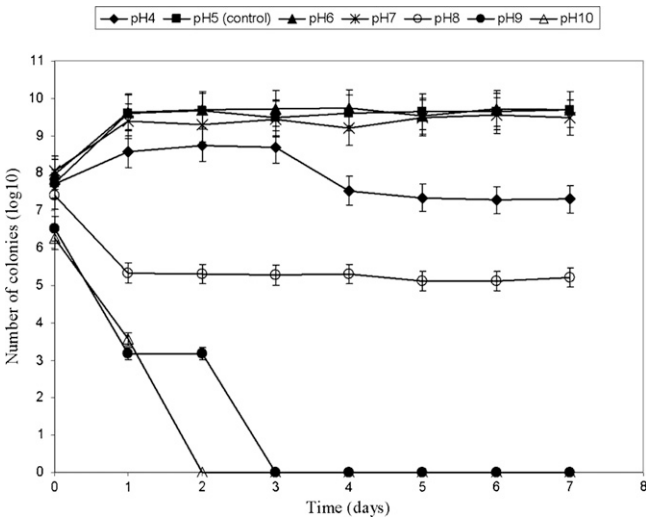

FIGURE 1. Effect of pH and incubation time on the survival of *B. pseudomallei* (log CFU/mL) in the soil microcosm at pH 4–10. CFUs per milliliter were determined as the colony counts on the modified Ashdown's agar using the dropped plate method at 37°C for 7 days. Points show means ($N = 6$); vertical lines denote SD.

TABLE 2

Two-way ANOVA analysis of the effect of soil pH, salinity, iron, and C/N on the growth of *B. pseudomallei* in soil microcosm.

Source	Sum of squares	Degrees of freedom	Mean square	*F* value	Significance
Soil pH	3,786.543	6	631.091	109,986.327	0.000*
Time	103.282	7	14.755	2,571.432	0.000*
Soil pH × time	475.718	42	11.327	1,974.001	0.000*
Error	1.607	280	0.006		
Corrected total	4,367.150	335			
Salinity	2,946.155	7	420.879	69,897.554	0.000*
Time	134.350	7	19.193	3,187.453	0.000*
Salinity × time	725.447	49	14.805	2,458.747	0.000*
Error	1.927	320	0.006		
Corrected total	3,807.879	383			
Iron	21.744	4	5.436	258.438	0.000*
Time	101.036	7	14.434	686.214	0.000*
Iron × time	4.046	28	0.145	6.870	0.000*
Error	4.207	200	0.021		
Corrected total	131.032	239			
C/N ratio	245.687	5	49.137	5,621.283	0.000*
Time	223.146	7	31.878	3,646.812	0.000*
C/N ratio × time	118.532	35	3.387	387.428	0.000*
Error	2.098	240	0.009		
Corrected total	589.463	287			

*Statistically significant ($P \leq 0.05$).

ANOVA (Table 2). At lower soil pH (pH 4), the number of bacterium was not significantly different from the innoculation at day 0 after the study period although a slightly increase at the first three days was observed. The basic soil pH significantly suppressed the bacterial growth, because the number of *B. pseudomallei* drastically dropped from 10^8 to 10^5 CFU/mL at soil pH 8 and 10^3 CFU/mL at soil pH 9 and 10 within 1 day (Figure 1). Soil pH 8–10 could inhibit the growth of *B. pseudomallei* significantly ($P < 0.05$) (Table 3). The numbers of bacteria gradually reduced to below the level of detection within 2 and 3 days for pH 10 and 9, respectively. However, at pH 8, the bacteria could persist until the end of experiment.

Effect of salinity on the growth of *B. pseudomallei*. *B. pseudomallei* could grow in soil microcosms without significant difference ($P > 0.05$) from the control (0% NaCl) up to 0.5% NaCl. However, NaCl concentrations of higher than 1.0% affected the growth of this bacterium (Figure 2). At 0–0.5% NaCl, the numbers of bacteria increased from 10^8 to 10^{10} CFU/mL within 1 day and remained at this level until the end of experiment. At 0.7% NaCl, the number of *B. pseudomallei* slightly reduced, but the growth from initial inoculum could still be detected. *B. pseudomallei* could not grow in soil with NaCl over 0.7%. At 1% NaCl, the number of bacteria remained the same (10^8 CFU/mL) for 3 days and then reduced to 10^6 CFU/mL. Soil salinity at 2.5% and 5.0% could inhibit the growth of *B. pseudomallei*. The numbers of bacteria were quickly reduced from 10^8 CFU/mL to below the level of detection within 4 and 5 days at soil salinity of 5% and 2.5%, respectively. Table 3 shows Duncan's multiple range tests of the treatments at different concentrations.

Effect of iron on the growth of *B. pseudomallei*. *B. pseudomallei* growth in soil microcosm was enhanced after incubation for 1 day under the presence of all levels of iron concentrations. Notably, the presence of iron at 125 and 150 mg/kg significantly ($P < 0.05$) increased *B. pseudomallei* growth in soil microcosm compared with the control (Figure 3). The number of bacteria reached the steady stage within 2 or 3 days and remained stable until the end of the experiment.

Effect of C/N ratio on the growth of *B. pseudomallei*. In general, bacterial growth was enhanced with increase of organic substrate concentration but adversely affected by wider C/N ratios. In this study, *B. pseudomallei* growth was examined under the C/N ratio ranging from 10 to 40 for 7 days.

TABLE 3

Duncan's multiple range tests for the effect of soil pH, salinity, iron, and C/N ratio on growth and CFUs of *B. pseudomallei* in the soil microcosm during the first 0–7 days at 37°C

		Number of colonies (log 10)		
Factors	*N*	Minimum	Maximum	Mean
Soil pH				
4	48	7.0414	8.7993	7.886556*
5 (control)	48	7.6532	9.7853	9.374871†
6	48	7.9085	9.8261	9.460210‡
7	48	7.8976	9.6335	9.225175§
8	48	5.0128	7.5441	5.498369¶
9	48	0	6.6128	1.607606‖
10	48	0	6.3617	1.229073**
Total	336			
Salinity (% NaCl)				
0.0 (control)	48	7.5185	9.7924	9.313283*
0.25	48	7.6128	10.3304	9.688585†
0.4	48	7.5635	10.0792	9.561938‡
0.5	48	7.6021	9.8513	9.419521§
0.7	48	7.5185	9.4472	8.847806¶
1.0	48	5.8675	7.7538	6.805060‖
2.5	48	0	7.6767	3.149660**
5.0	48	0	7.6646	2.581748††
Total	384			
Iron (mg/kg)				
50 control)	48	7.0086	9.4955	8.828798*
75	48	7.4314	9.4771	8.920146*
100	48	7.4771	9.5441	9.062283†
125	48	7.4914	9.9031	9.524765‡
150	48	7.5185	9.9395	9.539894‡
Total	240			
C/N ratio				
10 (control)	48	7.7160	11.5051	10.050983*
15	48	7.6812	11.8062	10.126879†
20	48	7.6335	11.6628	10.112454†
25	48	7.6435	11.1461	9.925965‡
30	48	7.5051	10.0492	9.018746§
40	48	6.5051	9.6021	7.592454¶
Total	288			

The number of colonies is from three replicates of two sets of independent experiments (total sample size = 6). Different footnotes represent significant difference.

*Soil pH 4 significant difference from pH 5, 6, 7, 8, 9 and 10; Salinity 0.0% NaCl significant difference from 0.25, 0.4, 0.5, 0.7, 1.0, 2.5 and 5.0% NaCl; Iron 50 and 75 mg/kg significant difference from 100, 125 and 150 mg/kg; C/N ratio 10 significant difference from 15, 20, 25, 30 and 40.

†Soil pH 5 significant difference from pH 4, 6, 7, 8, 9 and 10; Salinity 0.25% NaCl significant difference from 0.0, 0.4, 0.5, 0.7, 1.0, 2.5 and 5.0% NaCl; Iron 100 mg/kg significant difference from 50, 75, 125 and 150 mg/kg; C/N ratio 15 and 20 significant difference from 10, 25, 30 and 40.

‡Soil pH 6 significant difference from pH 4, 5, 7, 8, 9 and 10; Salinity 0.4% NaCl significant difference from 0.0, 0.25, 0.5, 0.7, 1.0, 2.5 and 5.0% NaCl; Iron 125 and 150 mg/kg significant difference from 50, 75 and 100 mg/kg; C/N ratio 25 significant difference from 10, 15, 20, 30 and 40.

§Soil pH 7 significant difference from pH 4, 5, 6, 8, 9 and 10; Salinity 0.5% NaCl significant difference from 0.0, 0.25, 0.4, 0.7, 1.0, 2.5 and 5.0% NaCl; C/N ratio 30 significant difference from 10, 15, 20, 25 and 40.

¶Soil pH 8 significant difference from pH 4, 5, 6, 7, 9 and 10; Salinity 0.7% NaCl significant difference from 0.0, 0.25, 0.4, 0.5, 1.0, 2.5 and 5.0% NaCl; C/N ratio 40 significant difference from 10, 15, 20, 25 and 30.

‖Soil pH 9 significant difference from pH 4, 5, 6, 7, 8 and 10; Salinity 1.0% NaCl significant difference from 0.0, 0.25, 0.4, 0.5, 0.7, 2.5 and 5.0% NaCl.

**Soil pH 10 significant difference from pH 4, 5, 6, 7, 8 and 9; Salinity 2.5% NaCl significant difference from 0.0, 0.25, 0.4, 0.5, 0.7, 1.0 and 5.0% NaCl.

††Salinity 5.0% NaCl significant difference from 0.0, 0.25, 0.4, 0.5, 0.7, 1.0, and 2.5% NaCl.

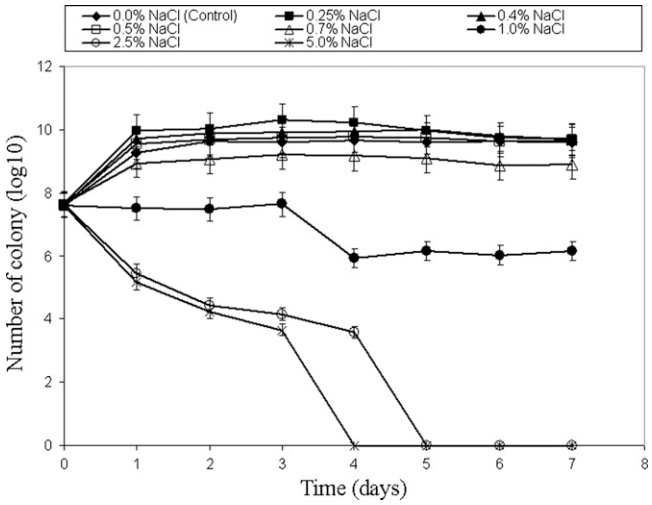

FIGURE 2. Effect of NaCl and incubation time on the survival of *B. pseudomallei* (log CFU/mL) in the soil microcosm at soil salinity 0.0% to 5.0% NaCl. CFUs per milliliter were determined as the colony counts on the modified Ashdown's agar using the dropped plate method at 37°C for 7 days. Points show means ($N = 6$); vertical lines denote SD.

The bacterial growth was increased at the C/N range from 10 to 25, but the number of *B. pseudomallei* significantly ($P < 0.05$) reduced at higher C/N ratio (40) at day 3 compared with the control (C/$N = 10$) (Figure 4).

DISCUSSION

Growth and distribution of *B. pseudomallei*, a soil-dwelling saprophytic bacterium, are influenced by environmental factors.[36] We first observed the effects of soil pH, soil salinity, iron concentration, and C/N ratio on the growth of *B. pseudomallei* in soil microcosms. Three physicochemical factors (pH, EC or salinity, and C/N) were the inhibitory

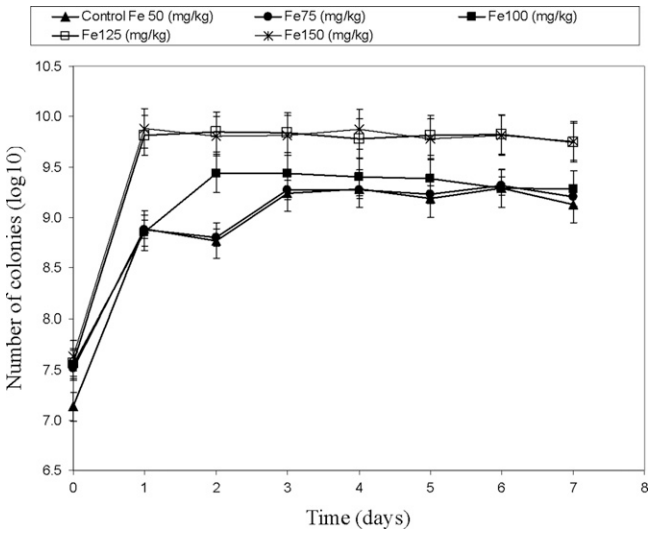

FIGURE 3. Effect of Fe and incubation time on the survival of *B. pseudomallei* (log CFU/mL) in the soil microcosm at total iron of 50–150 mg/kg. CFUs per milliliter were determined as the colony counts on the modified Ashdown's agar using the dropped plate method at 37°C for 7 days. Points show means ($N = 6$); vertical lines denote SD.

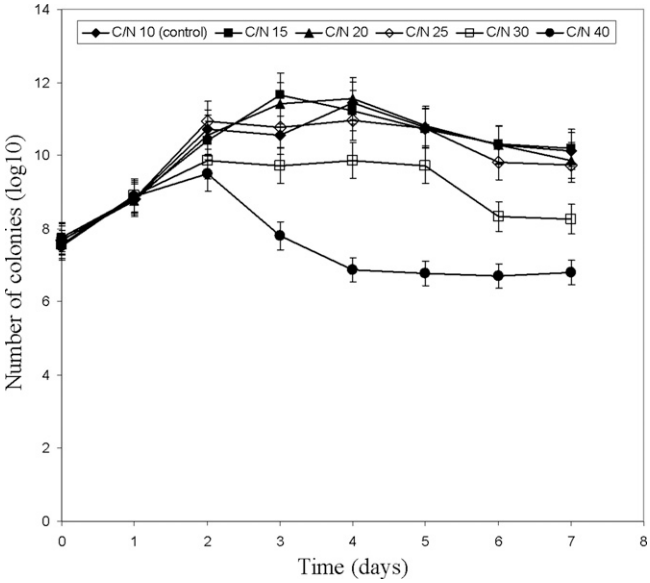

FIGURE 4. Effect of C/N ratio and incubation time on the survival of *B. pseudomallei* (log CFU/mL) in the soil microcosm at C/N ratios from 10:1 to 40:1. CFUs per milliliter were determined as the colony counts on the modified Ashdown's agar using the dropped plate method at 37°C for 7 days. Points show means ($N = 6$); vertical lines denote SD.

factors to the growth of *B. pseudomallei*, whereas Fe enhanced the number of bacteria.

Our results showed that *B. pseudomallei* could grow in the soil microcosm at pH 5–7. The bacterial growth was retarded slightly at pH 4 compared with the neutral or slightly acidic condition. Increase to soil pH 8 also caused significant ($P < 0.05$) reduction in the number of bacteria in the soil microcosm on the first day, and this reduction persisted during the time of the study. Notably, at pH 9–10, bacterial growth was inhibited, because the number of culturable *B. pseudomallei* went down drastically on the first day and disappeared within 3 days. The results from this study revealed that *B. pseudomallei* could survive in pH 4–7 in the soil microcosm, and they correlated with the growth study of the bacteria in soil medium (pH 5.5–8) by Chen and others,[29] which reported that the optimum soil pH for the growth of *B. pseudomallei* was 6.5–7.5. However, soil and *B. pseudomallei* in this study were collected from the same positive soil site in the endemic area to provide more realistic information. The bacterium was environmental and not a clinical isolate. Moreover, we studied the soil at a moisture content around 13%, reflecting the optimum range of terrestrial soil. Palasatien and others[25] also reported that *B. pseudomallei* remain stable in acidic soil (pH 5–6). Moreover, Tong and others[37] reported that *B. pseudomallei* grew well in broth at a pH range of 5.0–8.0. In addition, Dejsirilert and others[38] reported that these bacteria could persist in the medium at pH 4.5 for several months. The presence of *B. pseudomallei* in slightly acidic soil might, at least in part, account for the common distribution of this bacteria in rice fields in Thailand, where soil pH ranges from 4.4 to 7.7.[17] Because this bacterium prefers slightly acidic pH habitat but not basic soil pH, it might be possible to control *B. pseudomallei* by adjusting soil pH by adding lime into soil during the first tilling of the year. The excess lime will be washed out over the rainy season.

In this study, the number of bacteria in the soil microcosms with EC of 0.7% NaCl was significantly ($P < 0.05$) lower than the number in the soil microcosms with 0–5% NaCl. Notably, the number of viable *B. pseudomallei* was significantly lower at higher concentrations of NaCl (1%, 2.5% and 5% NaCl). However, the results showed that *B. pseudomallei* could tolerate high-salinity soil up to 4 days, and only the concentration higher than 2.5% could eradicate the bacteria completely. Inglis and Sagripanti[36] reported that *B. pseudomallei* could survive in NaCl solution at concentrations less than 2.5%.

According to the classification of salt-affected soil by the Department of Land Development of Thailand, normal or non-saline soil (0–2 dS/m) is the majority (65%) in the northeast of Thailand, including the soil sampling site. About 12% of the land in the northeast is slightly saline (2–4 dS/m or 0.65–0.95% NaCl), which can retard the growth of *B. pseudomallei* slightly at concentrations more than 2 dS/m. Only 5% of the land has salt concentration more than 4 dS/m (> 0.95% NaCl).[39] According to our result, only moderate and high-saline soil could completely inhibit *B. pseudomallei* growth. Moreover, because of the undulating terrain in the northeast of Thailand, the concentration of salt varies spatially, which might explain why the bacterium was found in a specific area but not isolated even few meters away.

It is possible to manage the number of *B. pseudomallei* in a small specific area where a high incidence of cases was reported[15–17] by adding salt to higher than 0.7% and then washing it out before cultivation. However, applying salt to soil may inhibit plant growth[40] and reduce productivity because of the osmolarity and elemental toxicity (for example, rice grown in moderately saline soil produced about a 20% yield loss).[41] However, *B. pseudomallei* recovered from stress conditions in soil microcosm using only colony formation may give the wrong impression about the survival of the bacteria, because stressed bacteria are considered viable but non-culturable cells (VBNCs),[36] which can still be responsible for cases of infectious diseases in humans. The other methodologies, such as flow cytometry and molecular techniques, may provide useful data in relation to the state transitions regarding cell *B. pseudomallei* viability, especially in environments.[36]

C/N ratio is an important factor, and a wider C/N ratio is not appropriate for saprophytic bacterium to degrade organic matter.[42] The growth of *B. pseudomallei* was increased during the first 2 or 3 days of the experiments and remains steady for a short period before slowly declining. At the widest C/N of C/N = 40, the number of bacteria slightly reduced to approximately 10^6 CFU/mL. Statistical analysis in Table 2 shows that C/N could significantly ($P < 0.05$) affect the growth of *B. pseudomallei*. However, only C/N = 40 had minor inhibitory impact on the bacterium growth. It may be because of the suppression on polyhydroxybutyrate (PHB) activity.[36] The accumulation of prominent granules of PHB as energy stores in bacillus reflects a metabolism adapted to long-term survival.[36]

It should be noted here that the purpose of changing soil physicochemical factors is to reduce the number of bacteria to a low limit threshold that is not enough to infect farmers. Three factors (pH, EC or salinity, and C/N) serve this purpose. In contrast, iron at all concentrations enhanced the growth of *B. pseudomallei* in soil microcosms, because iron is an essential microelement. Increase of iron concentration increased the growth of *B. pseudomallei* at the range of 50–150 mg/kg soil dry weight. Moreover, a concentration of iron higher than 125 mg/kg significantly increased bacterial growth ($P < 0.05$). Draper and others[43] reported that *B. pseudomallei* could be isolated in tap water containing 2–4 mg Fe/L. Yang and others[44] revealed that *B. pseudomallei* preferred to grow in a medium with the presence of $FeSO_4$. Kaestli and others[45] also mentioned that the presence of iron in soil in Australia favored the survival of *B. pseudomallei*. However, because there is a lot of genetic diversity in these organisms, the investigations of certain soil physicochemical properties on multiple *B. pseudomallei* isolate survival would be essential to determine the effectiveness of potential control measures.

In summary, the data showed that the bacterium was very resistant to extreme environments. Although a pH of 8 could reduce the number of bacteria, they may still persist in soil in non-culturable form. Similarly, the number of *B. pseudomallei* colonies was reduced at NaCl higher than 1.0%, but they are still able to survive at this concentration for the period examined. Although changing these soil factors could not completely wipe out the bacteria, the number of bacteria could be possibly reduced or suppressed at deeper soil horizon and will have a smaller chance of contacting farmers. In contrast, soil iron promoted the bacterial growth at all concentrations. Because the iron concentration in the soil of the endemic areas is generally very high, it might be, however, difficult to control the bacterium by controlling the iron level in the soil.

Because we have analyzed the effects of each physicochemical parameter separately, combined effects of two or more parameters should be explored in the future for better control of the bacteria.

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