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Physicochemical and Biological Factors of Soil and the Potential Use of Antagonistic Microbes for Biocontrol of *Burkholderia pseudomallei*

Rasana W. Sermswan [1,2] · Surasakdi Wongratanacheewin [2,3]

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Abstract
Purpose of Review The physicochemical properties of soil that are correlated with the presence of *Burkholderia pseudomallei* have been reported with controversial results. The knowledge of microbes with potential antagonistic effects against *B. pseudomallei* as biocontrol agents is also limited. This review therefore describes the knowledge of abiotic and biotic factors correlated with the presence or absence of *B. pseudomallei* and the potential use of microbes as a biocontrol.
Recent Findings The physicochemical factors in soil in unused land, rice paddies, or animal farms could identify significant factors correlated with the presence or absence of *B. pseudomallei*. The complex interaction of microbes and physicochemical factors may explain the uneven distribution of *B. pseudomallei* in soil. The potential use of bacteriophages and other antagonistic bacteria to control this lethal bacterium is discussed.
Summary Water content and acidic pH were two physicochemical factors commonly found correlating with the presence of *B. pseudomallei* in soil. Bacteriophages and some antagonistic bacteria showed potential to be used as biocontrols for *B. pseudomallei.*

Keywords *B. pseudomallei* · Physicochemical factors · Bacteriophages · *B. amyloliquefaciens* · Biocontrol · Soil pathogen

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✉ Surasakdi Wongratanacheewin
sura_wng@kku.ac.th

1 Department of Biochemistry, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand

2 Melioidosis Research Center, Khon Kaen University, Khon Kaen 40002, Thailand

3 Department of Microbiology, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand

Introduction

Burkholderia pseudomallei is a saprophytic bacterium found in the soil and water of its endemic area [1]. The cause of infection is mostly from contamination by the microbes through skin abrasion and less commonly from inhalation or ingestion [1]. Therefore, constituents of the environment create an important reservoir for this bacterium. Soil is the most complex biomaterial on the planet, containing a wide variety of interacting microbes to build their unique communities. The mechanisms for their survival in ecological environments include adherence, production of toxic metabolites, secretion of antimicrobial substances, and competition for nutrients [2]. Understanding the interference of such mechanisms that provide balance to each ecosystem will be beneficial for selecting antagonistic organisms against human or plant pathogens to be used for controlling microbes and lead to benefits for human and animal health as well as economics as a whole. Transmission of food-borne human pathogens from soil and the environmental spreading of infectious diseases are still major public health problems in several countries [3•]. Among the strategies currently used to control and prevent such infectious diseases, the use of living organisms to control other organisms (biocontrol) has been used for a long time especially for food-borne pathogens [4–7, 8]. In addition, plant pathogens from soil are another target to which biocontrol can be and has been applied [9–11]. As mentioned earlier, examples of biocontrol are already reported to control pathogens in food or soil using natural antagonists such as another bacteria or virus of the bacteria (bacteriophages) [12, 13].

A bacteriophage is a bacterial virus that acts as an obligate parasite. It is generally highly specific and strictly against a single bacterial strain; however, some bacteriophages have a broad host range [14]. The lytic bacteriophages are usually the main phages that are used to control bacteria. The obstacles of bacteriophages as biocontrol agents are as follows: firstly, some bacteriophages infect bacteria and incorporate their nucleic acid into the bacterial genomes, leading to a prophage which will not lyse the bacteria; secondly, as bacteriophages use bacteria as their host for propagation, the numbers of bacteria tend to be reduced but not eliminated by the phages; and lastly, some phages have broad host ranges and may affect other, perhaps beneficial, bacteria in the environment.

Microbes have been an important source of antibiotics for a long time because they can produce a wide spectrum of antimicrobial substances. Presently, several of them may still be a good source of antimicrobial peptides that can be used to fight against several kinds of organisms [15, 16]. They have been used successfully in controlling plant pathogens in soil and thus decrease the contaminated food-borne pathogens [17, 18, 19•]. Apart from those applications, biocontrol from environmental sources using bacteria against human diseases such as melioidosis is very limited.

This review article therefore discusses the soil environment of *B. pseudomallei* and is focused on the possible uses of bacteriophages and bacteria from the environment as a biocontrol of this disease-causing pathogen.

Environmental Factors and *B. pseudomallei*

The presence of *B. pseudomallei* in its ecological habitat was found to be correlated with some physicochemical factors in the environment in the endemic areas [20, 21, 22••, 23, 24••]. From soil surveys in two unused lands of the endemic area in Khon Kaen province, northeast Thailand, these surveys revealed the correlations of the bacteria with low soil pH (4.4–6), a low C/N (carbon/nitrogen) ratio of approximately 18, and a high extractable iron level of approximately 45 ppm [20, 21]. The studied areas are mostly sandy soil, and the organism was found mainly at the 5–30-cm depth. The endemic area of melioidosis disease in northeast Thailand was reported to be acidic [23], and this was confirmed to be one of the physicochemical factors that differed positive from negative soil sample areas. The results were correlated with information from the Agricultural Development Research Center (ADRC) in Thailand, which indicated that sandy soil covered 80% of northeast Thailand where acidic and infertile soils are common in that area. When 61 rice fields that covered the east, central, and northeast of Thailand were sampled at a 30-cm depth, however, the pHs and water contents were not significantly different between positive and negative soils for *B. pseudomallei* [24••]. The pHs of the soil varied ranging from 4.9 to 8.1, but the average pHs between the three regions were not significantly different. When taking a closer look at the average pH of soil in this study, pH > 6 was found in both *B. pseudomallei*-positive and *B. pseudomallei*-negative soils. The average water content was > 10% in both positive and negative soils that were reported to correlate with the presence or absence of the bacterium. Moreover, the types of soil in these regions were different so that the average of all samples may have altered the interpretation of factors correlated with the presence of the bacterium. A study of rice fields in Laos showed a majority of sandy soil types and acidity similar to the northeast of Thailand, but the bacteria predominated in a soil depth of more than 30 cm [25]. This seemed to be related to high moisture content and low total nitrogen and carbon [25]. Again, when the C/N ratio was calculated, both positive and negative soils showed a low C/N ratio of approximately 8–9. This finding goes together with other studies that showed that a low C/N ratio is correlated with the presence of the bacterium [19•, 20] and an increased ratio of > 40:1 could suppress the growth of *B. pseudomallei* [26]. Other factors such as chloride, organic carbon, phosphorus, potassium, manganese, or exchangeable cations have also been studied but did not show any significant effects when studied in farmlands and between the high and low prevalence areas on Castle hill in Townsville, Australia [22••, 27•]. As the culture of the bacteria is difficult to achieve together with the presence of the unculturable form of the bacteria in the soil [21, 28], negative cultivation may be misleading and variable results, therefore, could be obtained. Molecular methods have been suggested to be used for detecting the presence of the bacteria in the physicochemical studies of soil [25]. According to the results of the molecular method of detection, the comparison between undisturbed sites and environmentally manipulated areas in Australia for the habitat of *B. pseudomallei* indicated a positive association of *B. pseudomallei* in rich soils in grassy areas at undisturbed sites while the presence of livestock and a lower pH of soil were associated with manipulated areas [29]. The authors also concluded that *B. pseudomallei* may spread due to the changes in land management. On the whole, the outcome of factors studied in rice fields indicated a correlation of *B. pseudomallei* with nutrient depletion [24••, 27•] while high-manure areas with a decrease in C/N ratio and easy-to-digest carbon sources for microbes in farmland were also associated with the presence of the bacteria [22••]. *B. pseudomallei* is a bacterium that can tolerate diverse conditions from dry, sandy, and infertile soils to wet, clay, and high-manure soils. Therefore, comparisons of factors associated with the presence of the bacterium in different types of soil, different land usage of either undisturbed or manipulated areas, and different depths of water tables in each study area or country result in controversial results of physicochemical properties of soil. A soil survey in rice fields in the northeast of Thailand was reported to be 28% positive for *B. pseudomallei* with an average of 700 cfu while the empty areas were 80% positive with a 378 cfu [30]. Soil from farmlands disturbed with manure and rice fields that were loaded with fertilizers and tillage during cultivation could affect the ecosystem of the soil and consequently affect the physicochemical properties of the soil. Soil microcosm experiments to simulate various combinations of physicochemical factors in different soil types were conducted and resulted in data that were different from the environmental study [31••]. The combinations of pH, NaCl, iron and carbon-to-nitrogen ratios (C/N) on viability of bacteria cells in the developed microcosms showed that the moisture of soil, pH > 8, NaCl > 1%, and C/N ratios > 40:1 significantly reduced the viable bacteria while increasing the concentrations of iron significantly increased the bacterial growth [26]. Iron in soil is another controversial factor found in several studies as mentioned by Manivanh et al. [25]. The extractable iron (Fe^{2+}) is the reduced form that microbes can use and that was increased under acidic environment. Extractable iron may then be a better indicator than total iron to avoid misinterpretation in an environmental study. The details of various physicochemical factors effecting to the presence *B. pseudomallei* in the soil are shown in Table 1.

Therefore, to identify the physicochemical factors correlated with the presence of *B. pseudomallei* in soil in future studies, investigators should avoid collection of a large quantity of soil or composites of soil samples that may lead to misleading conclusions. Moreover, interpretations of each factor should not only reflect increases or decreases or correlations or negative correlations with the bacterium presence, but rather the range of each factor as low or high during the correlation analysis. Comparisons or interpretation of these factors between undisturbed and natural manipulated areas should also be performed with care.

The concept of using physicochemical factors to inhibit or suppress the bacterial growth or viability of the cells for controlling the bacteria is still difficult to apply due to the involvement of large and different usage areas, soil complexity, and differences in each ecosystem of both planted species and the soil community that always needs optimization [27•]. From all of those studies, it can be hypothesized that the use of physiochemical factors to either support or inhibit the *B. pseudomallei* and result in controlling the bacterium is unlikely, but information concerning these factors should help the biocontrol of the disease in soil.

Biological Factors and *B. pseudomallei*

Several reports demonstrated that abiotic factors in soil could affect the presence or absence of *B. pseudomallei* [20, 21, 22••, 24••]; however, only a few factors were significantly involved and those biotic factors were not included in the studies. Bacteriophages (phages) [34–36] and other soil saprophytic bacteria [37] were found to be present in the same

Table 1 Physicochemical factors that affect the growth of *B. pseudomallei* in soil

Physicochemical factors	Inhibit or suppress or eliminate *B. pseudomallei*	Increases or support presence of *B. pseudomallei*	References
pH	3.7–5.0 or > 8.0 Low or high pH	5.0–6.0 6.5 or 7.5	[20, 21, 26, 32]
Salt	> 1–1.5% NaCl	ND	[21, 33]
C/N ratio	> 40:1	Low C/N ratio	[21, 26]
Iron[a]	98.91 ± 18.36 mg/L 160 mg/kg	247.00 ± 42.08 mg/L 13 mg/kg	[21, 22••, 27•]
% Moisture content[a]	8.24 ± 0.80	> 10% 11.08 ± 0.74	[20, 22••]
Chemical O_2 demand	ND	High	[20]
Gravimetric water	15.7%	7.4%	[27•]
Nitrate/nitrogen	7 mg/kg	1.8 mg/kg	[27•]
Sulfur	20.5 mg/kg	2.4 mg/kg	[27•]
Exchangeable potassium	2.5%	8.2%	[27•]
Copper	4.4 mg/kg	0.24 mg/kg	[27•]
Zinc	40 mg/kg	4.8 mg/kg	[27•]
% Clay content[a]	5.13 ± 0.58	9.43 ± 1.61	[27•]

ND not determined

[a] Result expressed as mean ± SE

ecosystems as *B. pseudomallei* and showed ability to lyse the bacterium. Phages that could infect *B. pseudomallei* were also found in soil with the absence of the bacterium indicating the possibility that the phages were affecting the density of this bacterium in the environment [38••]. Phages can act by integrating their DNA into the host genome and cause damage by infecting, multiplying, and lysing the host cell at the end of its propagation. The first lytic bacteriophage for *B. pseudomallei* was isolated from stagnant water in Hanoi in 1956, but it was not characterized [39]. Later, several groups of researchers isolated and characterized *B. pseudomallei* phages from the soil environment [36, 37, 38••, 39, 40•]. The ST79 lytic phage, isolated from soil in Khon Kaen, Thailand, gave clear lytic plaques on *B. pseudomallei* lawn [36]. The lysis cassettes, which the enzymes used to lyse *B. pseudomallei*, are composed of a holin, a peptidase M15A or endolysin, and the *lysB* and *lysC* genes [40•]. Both lytic and lysogenic phages of *B. pseudomallei* are summarized in Table 2. There are several prophage and prophage-like elements inside the *B. pseudomallei* genome [44] that may contribute to the genetic and phenotypic diversity of *B. pseudomallei*, while the lytic phages might be used to control the bacteria [42]. The presence of phages specific for *B. pseudomallei* may affect the distribution of pathogens in the soil [35]. As bacteriophages have been used for controlling various food pathogens [5, 45, 46], the use of lytic phages as a biocontrol for *B. pseudomallei* has therefore also been proposed [36, 40•]. One obstacle of this hypothesis, however, is that the balance of the bacteriophage life cycle with the host that may lead to not totally clearing the host- and phage-resistant bacteria [47] may be developed. The use of phage cocktails in other bacteria has been reported to overcome this problem [48, 49]. Modified phage derivatives of ST79 that are specific to *B. pseudomallei* were developed to increase the lysis ability and reduce the biofilm formation of *B. pseudomallei* [50]; however, regrowth of the bacterial host was observed after the lysis cycle [36], which emphasizes the problem that has to be overcome if this phage or phage cocktails will be used. Up until now, there is no published work showing the successful use of bacteriophages as biocontrols for *B. pseudomallei*. In addition to the bacteriophage, various genera of bacteria have been reported to be used as biocontrols such as *Agrobacterium*, *Bacillus*, and *Pseudomonas* [51, 52•, 53, 54]. Most of these are already approved to be used as commercial products. One example of a bacterium is *Bacillus amyloliquefaciens* [52•], which was successfully used as a biocontrol against plant pathogens. Biocontrol is more likely to be successful for some pathogens than others, and food-borne and plant pathogens are the two main groups where biocontrol is beneficial and for which products are available in the market [55, 56].

For melioidosis, in the last 10 years, only a few bacteria were reported to interfere or inhibit *B. pseudomallei*. The same genera of bacteria which are *Burkholderia ubonensis* [57] and *Burkholderia multivorans* [58] were shown to have some antagonistic effects on *B. pseudomallei*. *B. ubonensis* produces specific antagonistic compounds active against *B. pseudomallei* [57]. This compound was found to be a pepsin-sensitive peptide similar to a bacteriocin-like compound that may be purified and characterized [57]. Another bacterium, *B. multivorans*, was discovered from soil [58]. The

Table 2 Bacteriophages that infect *B. pseudomallei*

Bacteriophages names	Type	Infectivity or from	Potential use for biocontrol	References:
Unknown	Lytic phage	*B. pseudomallei*	Unknown	[39]
Φ1026b	Prophage	*B. pseudomallei*	No	[41]
ST2	Lytic phage	78% *B. pseudomallei*, 71% *B. thailandensis*, *B. mallei*	Yes	[36]
ST7	Lytic phage	41% *B. pseudomallei*, *B. mallei*	Yes	[36]
ST70	Lytic phage	65% *B. pseudomallei*, *B. mallei*	Yes	[36]
ST88	Lytic phage	41% *B. pseudomallei*, *B. mallei*	Yes	[36]
ST96	Lytic phage	67% *B. pseudomallei*, 71% *B. thailandensis*, *B. mallei*	Yes	[36]
ST79	Lytic phage	71% *B. pseudomallei*, *B. mallei*	Yes	[36, 40•]
φ52237	Prophage	*B. pseudomallei*	No	[42]
φ644-2	Prophage	*B. pseudomallei*	No	[42]
φE12-2	Prophage	*B. pseudomallei*	No	[42]
φBp-AMP1	Lytic phage	*B. pseudomallei*, *B. thailandensis*, *B. multivorans*, *B. ubonensis*, *B. vietnamensis*, *B. cephacia*, *Pseudomonas aeruginosa*, *Escherichia coli*	Yes	[34]
Φ1026b	Prophage	*B. pseudomallei*	No	[41]
φX216	Temperate phage	78% of *B. pseudomallei*, *B. mallei*	No	[43]

antagonist effect was demonstrated by the presence of *B. multivorans* and was inversely related to the presence of *B. pseudomallei* [58]. The antagonistic activity of secreted compounds from this bacterium was also reported. As *B. multivorans* was found in the same area of *B. pseudomallei* and is more resistant to a broad range of temperature, pH, and salt than *B. pseudomallei*, the possible use of it as a biocontrol has therefore been discussed. These two bacteria, however, are human pathogens; therefore, the use of *B. multivorans* for biocontrol of *B. pseudomallei* may not be possible.

Another candidate for biocontrol of *B. pseudomallei* is *B. amyloliquefaciens*, which was isolated from soil that was negative for *B. pseudomallei*. A few isolates of them have been reported to produce both peptides and non-peptide metabolites that can kill *B. pseudomallei* and inhibit a broad range of other pathogenic bacteria [37]. The inhibition spectrum was possible from the synergistic effect of the non-protein and peptide compounds. The picture highlighting the active compounds that gave large clear zones on *B. pseudomallei* culture lawns is shown in Fig. 1. When *B. amyloliquefaciens* isolates were cocultured with *B. pseudomallei*, the numbers of the *B. pseudomallei* bacteria decreased by 5 logs in 72 h [37]. *B. amyloliquefaciens* produces spores that can tolerate environmental changes and resist desiccation. These spores, when germinated, secrete metabolites that can kill *B. pseudomallei*, and therefore, it could be used as biological controls for *B. pseudomallei* in the environment.

The use of any microbes for controlling *B. pseudomallei* in soil should be applied when the soil is able to absorb large amounts of water such as during the rainy season. This will allow interaction of microbes and increase the possibility of biocontrol. The treatment should be continuous, and long-term observation is needed to see changes in the decreases of bacterium in soil due to biocontrol treatment.

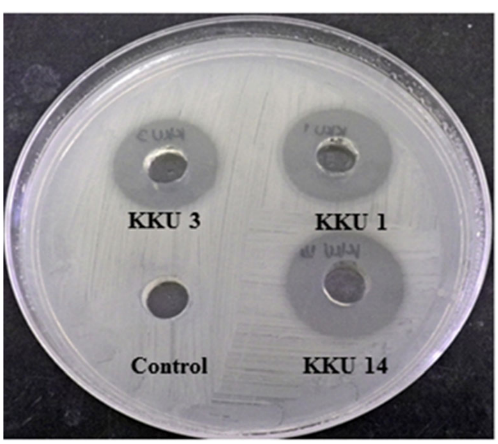

Fig. 1 Antimicrobial activity of *B. amyloliquefaciens* against *B. pseudomallei*. The inhibitory activity of culture supernatants from *B. amyloliquefaciens* KKU1, KKU3, and KKU14 isolates when tested against *B. pseudomallei* using the agar well diffusion method and is seen by clear zones. *MM* minimal medium (control)

Conclusion

Both abiotic and biotic factors in soil were proven to contribute to the presence and absence of *B. pseudomallei* and may also play a crucial role in providing an uneven distribution of the bacterium in soil. The discovery of bacteriophages and some antagonistic bacteria in soil which can secrete metabolites to inhibit or kill *B. pseudomallei* is a new research trend and gives potential for biocontrols to reduce the risk of infection when humans and animals are exposed to *B. pseudomallei* due to contact with contaminated soil and water in the endemic areas.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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