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Survival of *Burkholderia pseudomallei* on Environmental Surfaces[▽]

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The survival of the biothreat agent *Burkholderia pseudomallei* on the surfaces of four materials was measured by culture and esterase activity analyses. The culture results demonstrated that this organism persisted for <24 h to <7 days depending on the material, bacterial isolate, and suspension medium. The persistence determined by analysis of esterase activity, as measured with a ScanRDI solid-phase cytometer, was always longer than the persistence determined by culture analysis.

Burkholderia pseudomallei is a gram-negative bacillus that is the causative agent of melioidosis, a disease whose severity varies and which is often difficult to treat and has a mortality rate that can be as high as 60 to 70% (4, 7, 10, 12, 14, 17). *B. pseudomallei* is endemic in Northern Australia and Southeast Asia but can survive in areas outside its natural habitat (12). Documented cases have occurred in France, the United States, and elsewhere (8, 12, 15, 16, 27). *B. pseudomallei* is currently considered a biothreat agent; thus, knowledge about its persistence on specific surfaces is important in order to help with risk assessment, sampling, and decontamination strategies. Previous studies have focused on the survival of *B. pseudomallei* in its natural environment (wet areas with high temperatures [37 to 42°C]) and on the effect of different environmental factors, such as pH, water content, and temperature, in a laboratory setting (3, 4, 7, 8, 10, 12, 14, 17, 19, 20, 26, 27). To date, there have been no data related to the survival of this organism in or on man-made environments.

The length of survival of two isolates of *B. pseudomallei* suspended in either Butterfield buffer (BB) or brain heart infusion broth (BHI) on glass, stainless steel (SS), paper, and polyethylene (PE) coupons was investigated. Since culture results have been shown to underestimate the number of viable organisms after exposure to stressful conditions, the viability of bacterial cells was also assayed by detecting esterase activity using ChemChrome V6 and a solid-phase cytometer (SPC) (ScanRDI; Chemunex, Ivy-Sur-Seine, France) (1, 5).

B. pseudomallei strains ATCC 23343 and ATCC 11668 were grown on Trypticase soy agar containing 5% sheep blood (TSA II; Becton, Dickinson and Company, Sparks, MD) at 35°C for 24 h. The test surfaces and the methods used for suspension preparation, coupon inoculation, humidity control, and cell recovery have been described elsewhere (18). Coupons were sampled periodically for at least 7 days, while some coupons were sampled for up to 56 days. Survival, as measured by the presence of esterase activity, was determined by using the ScanRDI sample preparation protocol (6) with an additional step (placement of the filter onto a pad saturated with 600 μl of 6% formaldehyde for 15 min) to kill the bacteria for safe transport and handling. Preliminary studies verified that the additional step did not adversely affect the assay results (data not shown).

The colony and cell count data were adjusted for dilution and volume. To account for some zeros, the data were modified and then transformed [$\log_{10}(x + 1)$]. At each sampling point, means and standard deviations were calculated. The normality of the data was tested using the Shapiro-Wilk statistic, and the mean values were compared to see if significant differences occurred over time and if there were significant differences between isolates, suspensions, and material types (a significant P value was a P value of ≤ 0.05) (25). The Student t test or the Wilcoxon rank sum test was used when appropriate to compare the means (SAS, version 9.1; SAS Institute, Cary, NC).

The lengths of survival for each isolate on each material and in the different suspensions are shown in Tables 1 to 4. The esterase activity analysis indicated longer survival times than the culture analysis for both isolates regardless of the suspension medium or surface material (Tables 1 to 4). With two exceptions (PE inoculated with ATCC 23343 in BB and SS inoculated with ATCC 11668 in BB), cells on all materials were still alive at 21 days as determined by esterase activity, whereas cells did not survive for more than 7 days as determined by culture analysis (Tables 1 and 3). At 24 h, there was a significant difference between the esterase and culture results for both isolates on all materials. An example of this is that the survival times for *B. pseudomallei* ATCC 23343 on paper determined by the esterase activity and culture analyses were not significantly different up to 2 h; however, there was a significant difference at ≥4 h (for BB, $P < 0.001$; for BHI, $P = 0.03$). The differences continued to be significant at 28 days, when they were >2 $\log_{10}$ cells/coupon in BB and >5 $\log_{10}$ cells/coupon in BHI. In all instances, esterase activity was detected for much longer periods of time than culture activity. Since culture analysis is the "gold standard" for determining the viability of bacteria, the results described below focus on the differences in culture data.

There was no significant difference in the number of culturable cells between the isolates suspended in BB on any material ($P \geq 0.05$) (Table 1). When cells were suspended in BHI

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TABLE 1. Recovery of *B. pseudomallei* isolates suspended in BB and placed on four materials as determined by culture analysis

Time after inoculation (h)	Mean $\log_{10}$ CFU/coupon (SD) on indicated surface ($n = 6$)							
	Paper		SS		Glass		PE	
	ATCC 23343	ATCC 11668	ATCC 23343	ATCC 11668	ATCC 23343	ATCC 11668	ATCC 23343	ATCC 11668
0	4.82 (0.07)	4.96 (0.19)	3.40 (0.23)	3.24 (0.35)	3.65 (0.31)	4.04 (0.47)	3.13 (0.08)	4.53 (0.19)
2	5.27 (0.33)	4.65 (0.03)	2.44 (1.41)	3.34 (0.44)	2.54 (1.41)	3.31 (0.22)	2.52 (0.16)	4.42 (0.07)
4	4.76 (0.11)	4.45 (0.11)	2.97 (0.33)	3.36 (0.12)	2.49 (1.32)	1.88 (1.48)	2.54 (0.23)	4.38 (0.09)
6	2.77 (0.47)	3.78 (0.46)	0.17 (0.50)	2.36 (1.40)	1.02 (1.58)	0.75 (0.68)	0.79 (1.23)	3.43 (1.60)
24	0.00 (0.00)	0.05 (0.12)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
72	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)

and placed on SS, the ATCC 11668 isolate could be cultured at 72 h, while the ATCC 23343 isolate could not ($P < 0.0001$) (Table 2). The ATCC 23343 isolate in BHI survived on PE until 72 h, while the ATCC 11668 isolate did not, although the difference was not significant ($P = 0.14$) (Table 2). Both isolates generally survived longer when cells were suspended in BHI than when cells were suspended in BB (Tables 1 and 2).

The isolates showed no growth as determined by culture analysis at 24 h when they were suspended in BB on any of the materials, with the exception of ATCC 11668 on paper. When the ATCC 11668 isolate was placed on paper, it survived longer than the ATCC 23343 isolate, although the difference was not significant ($P = 0.21$) (Table 1). When suspended in BHI, cells of both isolates were able to survive for 72 h on paper, and the survival times for ATCC 11668 on SS and ATCC 23343 on PE were comparable. In BHI, both isolates survived for at least 24 h on all surfaces, with the exception of ATCC 23343 on SS (Tables 1 and 2).

While plate counting is the traditional method of determining the presence of bacteria on a surface, the results often underrepresent the number of viable bacteria present (1, 5, 22). In a previous study looking at the effect of free chlorine on *B. pseudomallei* in water, it was noted that culture methods were unable to detect *B. pseudomallei* after treatment; however, persistent cells were still detected using flow cytometry and a membrane integrity assay (11). Our study confirmed that the SPC and the esterase viability assay could detect extended persistence of *B. pseudomallei* compared with traditional culture media, and this has been demonstrated to be true for other organisms as well (1, 5). Whether the cells entered a viable but not culturable (VBNC) state or were simply injured is not known, but *B. pseudomallei* is thought to enter a VBNC state in its natural environment in order to survive through the dry season (2, 11–13). The pathogenicity of these stressed *B. pseudomallei* cells is not yet known. Some *B. pseudomallei* isolates differ in virulence depending on the strain, iron availability, inoculum size, and host factors; thus, the pathogenicity of stressed *B. pseudomallei* strains may also differ (21).

When the two isolates were compared, the suspension medium influenced the survival of the isolates differently. The survival times of the two isolates suspended in BB were not significantly different, but the survival times were different when the isolates were suspended in BHI. This may indicate that when cells are suspended in a more complex medium (BHI), the specific properties of the surface materials affect the isolates differently with regard to resistance to desiccation. Suspension in a buffer solution like BB may not provide any protection from desiccation, and the surface properties of the materials could have a stronger influence, negating isolate differences. However, it has been noted that many bacteria are better able to resist disinfection with chemical agents when they are maintained in a low-nutrient medium, such as certain types of water (11). Culture analyses did not show this trend with drying, but the esterase assay results did indicate that BB and BHI allowed nonculturable cells to persist for similar amounts of time.

When both isolates were suspended in BHI, they dried more slowly, and the additional components of the BHI may have prevented the cell membrane from distorting while the cells were drying (18, 23, 24). For the SPC results, the presence of these additional components could also explain the slight increase in the number of cells with active esterase suspended in

TABLE 2. Recovery of *B. pseudomallei* isolates suspended in BHI and placed on four materials as determined by culture analysis

Time after inoculation (h)	Mean $\log_{10}$ CFU/coupon (SD) on indicated surface ($n = 6$)							
	Paper		SS		Glass		PE	
	ATCC 23343	ATCC 11668	ATCC 23343	ATCC 11668	ATCC 23343	ATCC 11668	ATCC 23343	ATCC 11668
0	6.29 (0.05)	6.17 (0.05)	5.43 (0.11)	5.74 (0.06)	4.73 (0.10)	5.81 (0.08)	5.77 (0.06)	5.81 (0.05)
2	6.11 (0.13)	6.12 (0.13)	4.91 (0.46)	5.69 (0.04)	4.42 (0.38)	5.61 (0.31)	5.81 (0.03)	5.74 (0.07)
4	6.02 (0.14)	6.07 (0.17)	5.37 (0.16)	5.43 (0.22)	3.59 (0.73)	4.68 (0.85)	5.37 (0.55)	5.83 (0.06)
6	5.32 (0.26)	5.77 (0.02)	4.39 (0.08)	4.80 (0.07)	3.19 (0.57)	3.75 (2.32)	4.79 (0.21)	5.77 (0.06)
24	3.29 (0.15)	1.99 (0.38)	0.00 (0.00)	3.41 (0.14)	0.10 (0.25)	0.22 (0.35)	2.78 (0.19)	2.36 (0.03)
72	0.10 (0.25)	0.10 (0.25)	0.00 (0.00)	1.25 (0.21)	0.00 (0.00)	0.00 (0.00)	0.20 (0.35)[a]	0.00 (0.00)
168	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)[a]	0.00 (0.00)

[a] $n = 3$.

TABLE 3. Recovery of *B. pseudomallei* isolates suspended in BB and placed on four materials as determined by esterase activity analysis

Time after inoculation (h)	Mean $\log_{10}$ cells/coupon (SD) on indicated surface ($n = 3$)							
	Paper		SS		Glass		PE	
	ATCC 23343	ATCC 11668	ATCC 23343	ATCC 11668	ATCC 23343	ATCC 11668	ATCC 23343	ATCC 11668
0	4.88 (0.08)	6.06 (1.31)	5.11 (0.35)	3.04 (2.71)	4.53 (1.34)	0.00 (0.00)[a]	4.77 (0.36)	4.59 (0.22)
2	5.30 (0.03)	4.38 (0.29)	2.75 (2.41)	3.10 (0.11)	3.31 (1.19)	3.27 (0.25)	4.18 (0.18)	4.54 (0.05)
4	4.31 (0.11)	4.17 (0.13)	3.58 (0.56)	3.57 (0.08)	3.67 (0.65)	2.60 (0.45)	3.98 (0.21)	4.44 (0.09)
6	4.18 (0.32)	3.80 (0.18)	2.72 (0.75)	3.95 (0.14)	2.86 (0.83)	1.79 (0.21)	1.81 (1.68)	3.37 (0.44)
24	4.36 (0.21)	3.76 (0.15)	1.77 (0.31)	1.82 (0.07)	1.66 (0.37)	1.56 (0.55)	0.37 (0.64)	2.31 (0.54)
72	3.94 (0.82)	3.38 (0.02)	1.35 (0.13)	0.33 (0.58)	1.41 (0.60)	2.24 (0.63)	1.16 (0.41)	2.00 (0.46)
168	3.36 (0.37)	3.61 (0.17)	0.40 (0.70)	0.00 (0.00)	0.80 (0.70)	1.18 (0.56)	0.20 (0.35)	2.26 (0.78)
336	3.18 (0.84)	3.67 (0.23)	0.43 (0.74)	0.25 (0.43)	0.72 (0.79)	0.25 (0.43)	0.00 (0.00)	1.70 (0.31)
504	2.88 (0.77)	2.71 (0.68)	0.33 (0.58)	ND[b]	1.43 (0.60)	0.73 (0.72)	ND	1.33 (1.16)
672	2.45 (0.52)	2.17 (0.38)	ND	ND	ND	1.62 (0.65)	ND	ND
840	ND	2.72 (0.46)	ND	ND	ND	ND	ND	ND
1,008	ND	1.89 (0.22)	ND	ND	ND	ND	ND	ND

[a] Outlier due to sampling error which was not included in the statistical analysis.
[b] ND, not determined.

BHI starting around day 14 (Table 4). A similar increase was seen by Wuthiekanun et al. and was attributed to the dying cells providing enough nutrients for replication to occur in the surviving cells (26). Dimick observed something similar with *Serratia marcescens* and described it as a rhythmic response to cell age and environmental shifts that cause stress to the cells (9). The cells that survived may have been able to use the BHI nutrients and the dying cells to revive and reproduce before they started to decline again. In a previous study of the survival of *Yersinia pestis* on the same materials, it was noted that surface characteristics, such as free energy, electrostatic charge, roughness, and contact angle, may have influenced the length of survival (18). *B. pseudomallei*, like *Y. pestis*, generally survived for longer periods of time on paper, especially if it was suspended in BHI. It was noted by Rose et al. that paper was the roughest and most hydrophobic of the materials used, and this may have contributed to the longer survival of *B. pseudomallei* on paper in BHI (18). Components in the paper sizing may also have provided the bacteria with an additional source of nutrients.

In general, it appears that the specific properties of each material (roughness and hydrophobicity) affect the length of survival in a manner similar to that described for *Y. pestis* by Rose et al. (18), with glass being the least hospitable and paper, SS, and PE being similarly hospitable for *B. pseudomallei* survival. Survival on PE and SS was variable due to the isolate and the suspension medium. Suspension in BHI extended survival, possibly due to the addition of nutrients and protection from drying. Determination of viability by analysis of esterase activity demonstrated that the survival time of cells was extended compared to the results of culture analysis, raising the possibility that *B. pseudomallei* enters a VBNC state when it is stressed. Culture analysis may not be the most sensitive method for assessing the extent of a public health threat, and alternative methods to determine viability should also be used to assess the viability of organisms in environmental samples (1). The pathogenicity of *B. pseudomallei* in a possible VBNC state after desiccation stress is unknown and needs to be investigated to better determine the threat of this organism to public health.

TABLE 4. Recovery of *B. pseudomallei* isolates suspended in BHI and placed on four materials as determined by esterase activity analysis

Time after inoculation (h)	Mean $\log_{10}$ cells/coupon (SD) on indicated surface ($n = 3$)							
	Paper		SS		Glass		PE	
	ATCC 23343	ATCC 11668	ATCC 23343	ATCC 11668	ATCC 23343	ATCC 11668	ATCC 23343	ATCC 11668
0	6.30 (0.04)	6.18 (0.04)	4.92 (0.20)	5.81 (0.03)	5.80 (0.05)	5.88 (0.09)	5.85 (0.15)	5.92 (0.12)
2	6.21 (0.02)	6.15 (0.08)	5.43 (0.15)	5.61 (0.07)	4.95 (0.54)	5.81 (0.28)	5.90 (0.05)	5.86 (0.03)
4	6.21 (0.06)	6.06 (0.17)	5.43 (0.15)	5.56 (0.07)	4.20 (0.96)	4.94 (0.79)	5.61 (0.07)	5.88 (0.06)
6	5.85 (0.07)	5.85 (0.04)	3.28 (0.49)	4.90 (0.07)	4.03 (0.39)	3.69 (0.94)	5.82 (0.08)	6.01 (0.16)
24	5.85 (0.08)	5.04 (0.58)	3.90 (0.05)	4.42 (0.21)	1.60 (0.42)	2.16 (0.51)	4.24 (0.06)	4.20 (0.22)
72	5.65 (0.04)	4.50 (0.39)	3.04 (0.39)	4.39 (0.16)	2.98 (0.21)	2.50 (0.42)	3.67 (0.33)	4.10 (0.14)
168	5.56 (0.20)	3.96 (0.37)	3.10 (0.41)	2.24 (0.29)	1.95 (0.25)	2.09 (0.42)	3.52 (0.07)	3.67 (0.31)
336	5.01 (0.07)	3.74 (0.16)	3.54 (0.22)	1.69 (0.27)	2.05 (0.49)	2.93 (0.75)	2.11 (0.60)	2.13 (0.11)
504	5.36 (0.03)	3.75 (0.16)	2.47 (0.17)	2.71 (0.27)	1.22 (0.10)	2.21 (0.68)	3.74 (0.19)	4.42 (0.14)
672	4.97 (0.64)	3.70 (0.06)	2.29 (0.55)	2.87 (0.24)	1.70 (0.28)	2.05 (0.14)	3.64 (0.16)	4.40 (0.14)
840	4.58 (0.33)	3.68 (0.09)	3.88 (0.48)	2.67 (0.08)	2.04 (0.39)	2.01 (0.32)	3.78 (0.16)	4.32 (0.02)
1,008	4.44 (0.38)	2.88 (0.17)	ND[a]	2.86 (0.26)	ND	ND	3.85 (0.10)	4.21 (0.20)
1,176	4.96 (0.15)	3.36 (0.08)	ND	2.15 (0.11)	ND	ND	ND	4.48 (0.12)
1,344	4.87 (0.52)	3.24 (0.08)	ND	ND	ND	ND	ND	ND

[a] ND, not determined.

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REFERENCES

1. **Baudart, J., J. Coallier, P. Laurent, and M. Prévost.** 2002. Rapid and sensitive enumeration of viable diluted cells of members of the family *Enterobacteriaceae* in freshwater and drinking water. Appl. Environ. Microbiol. **68:**5057–5063.
2. **Bogosian, G., and E. V. Bourneuf.** 2001. A matter of bacterial life and death. EMBO Rep. **21:**770–774.
3. **Chen, Y. S., S. C. Chen, C. M. Kao, and Y. L. Chen.** 2003. Effects of soil pH, temperature and water content on the growth of *Burkholderia pseudomallei*. Folia Microbiol. **48:**253–256.
4. **Cheng, A. C., J. N. Hanna, R. Norton, S. L. Hills, J. Davis, V. L. Krause, G. Dowse, T. J. Inglis, and B. J. Currie.** 2003. Melioidosis in northern Australia, 2001-02. Commun. Dis. Intell. **27:**272–277.
5. **Cools, I., E. D'Haese, M. Uyttendaele, E. Storms, H. J. Nelis, and J. Debevere.** 2005. Solid phase cytometry as a tool to detect viable but non-culturable cells of *Campylobacter jejuni*. J. Microbiol. Methods **63:**107–114.
6. **Costanzo, S. P., R. N. Borazjani, and P. J. McCormick.** 2002. Validation of the Scan RDI for routine microbiological analysis of process water. PDA J. Pharm. Sci. Technol. **56:**206–219.
7. **Currie, B. J., D. A. Fisher, D. M. Howard, J. N. C. Burrow, S. Selvanayagam, P. L. Snelling, N. M. Anstey, and M. J. Mayo.** 2000. The epidemiology of melioidosis in Australia and Papua New Guinea. Acta Trop. **74:**121–127.
8. **Dance, D. A. B.** 2000. Ecology of *Burkholderia pseudomallei* and the interactions between environmental *Burkholderia* spp. and human-animal hosts. Acta Trop. **74:**159–168.
9. **Dimmick, R. L.** 1965. Rhythmic response of *Serratia marcescens* to elevated temperature. J. Bacteriol. **89:**791–798.
10. **Govan, B., and N. Ketheesan.** 2004. Exposure to *Burkholderia pseudomallei* induces cell-mediated immunity in healthy individuals. Eur. Soc. Clin. Microbiol. Infect. Dis. **10:**585–587.
11. **Howard, K., and T. J. J. Inglis.** 2003. The effect of free chlorine on *Burkholderia pseudomallei* in potable water. Water Res. **37:**4425–4432.
12. **Inglis, T. J. J., and J. Sagripanti.** 2006. Environmental factors that affect the survival and persistence of *Burkholderia pseudomallei*. Appl. Environ. Microbiol. **72:**6865–6875.
13. **Inglis, T. J. J., N. F. Foster, D. Gal, K. Powell, M. Mayo, R. Norton, and B. J. Currie.** 2004. Preliminary report on the northern Australian melioidosis environmental surveillance project. Epidemiol. Infect. **132:**813–820.
14. **Josephson, J.** 2001. Melioidosis: an emerging tropical health problem. Environ. Sci. Technol. **35:**455A–457A.
15. **McCormick, J. B., R. E. Weaver, P. S. Hayes, J. M. Boyce, and R. A. Feldman.** 1977. Wound infection by an indigenous *Pseudomonas pseudomallei*-like organism isolated from the soil: case report and epidemiologic study. J. Infect. Dis. **135:**103–107.
16. **Mollaret, H. H.** 1988. "L'affaire du Jardin des Plantes" ou comment la méioïdose fit son apparition en France. Med. Mal. Infect. **18:**643–654.
17. **Ngauy, V., Y. Lemeshev, L. Sadkowski, and G. Crawford.** 2005. Cutaneous melioidosis in a man who was taken as a prisoner of war by the Japanese during World War II. J. Clin. Microbiol. **43:**970–972.
18. **Rose, L. J., R. Donlan, S. N. Banerjee, and M. J. Arduino.** 2003. Survival of *Yersinia pestis* on environmental surfaces. Appl. Environ. Microbiol. **69:**2166–2171.
19. **Thomas, A. D., J. Forbes-Faulkner, and M. Parker.** 1979. Isolation of *Pseudomonas pseudomallei* from clay layers at defined depths. Am. J. Epidemiol. **110:**515–521.
20. **Tong, S., S. Yang, Z. Lu, and W. He.** 1996. Laboratory investigation of ecological factors influencing the environmental presence of *Burkholderia pseudomallei*. Microbiol. Immunol. **40:**451–453.
21. **Ulett, G. C., B. J. Currie, T. W. Clair, M. Mayo, N. Ketheesan, J. Labrooy, D. Gal, R. Norton, C. A. Smith, J. Barnes, J. Warner, and R. G. Hirst.** 2001. *Burkholderia pseudomallei* virulence: definition, stability and association with clonality. Microbes Infect. **3:**621–631.
22. **Wallner, G., D. Tillmann, and K. Haberer.** 1999. Evaluation of the ChemScan system for rapid microbiological analysis of pharmaceutical water. PDA J. Pharm. Sci. Technol. **53:**70–74.
23. **Webb, S. J.** 1960. Factors affecting the viability of air-borne bacteria. II. The effect of chemical additives on the behavior of air-borne cells. Can. J. Microbiol. **6:**71–87.
24. **Webb, S. J.** 1960. Factors affecting the viability of air-borne bacteria. III. The role of bonded water and protein structure in the death of air-borne cells. Can. J. Microbiol. **6:**89–105.
25. **Weiss, N. A.** 2002. Introductory statistics, 6th ed. Addison-Wesley, Boston, MA.
26. **Wuthiekanun, V., M. D. Smith, and N. J. White.** 1995. Survival of *Burkholderia pseudomallei* in the absence of nutrients. Trans. R. Soc. Trop. Med. Hyg. **89:**491.
27. **Yabuuchi, E., L. Wang, M. Arakawa, and I. Yano.** 1993. Survival of *Pseudomonas pseudomallei* strains in 5 degrees C. J. Jpn. Assoc. Infect. Dis. **67:**331–335.