

1 **SURVIVAL KINETICS IN 1/4 STRENGTH HOAGLANDS BROTH OVER SEVEN**  
2 **DAYS**

3 **INTRODUCTION**

4 Survival kinetics were carried out to identify any variable effect of bacterial growth or  
5 survival in Hoaglands agar which could confound the results of the plant experiment.

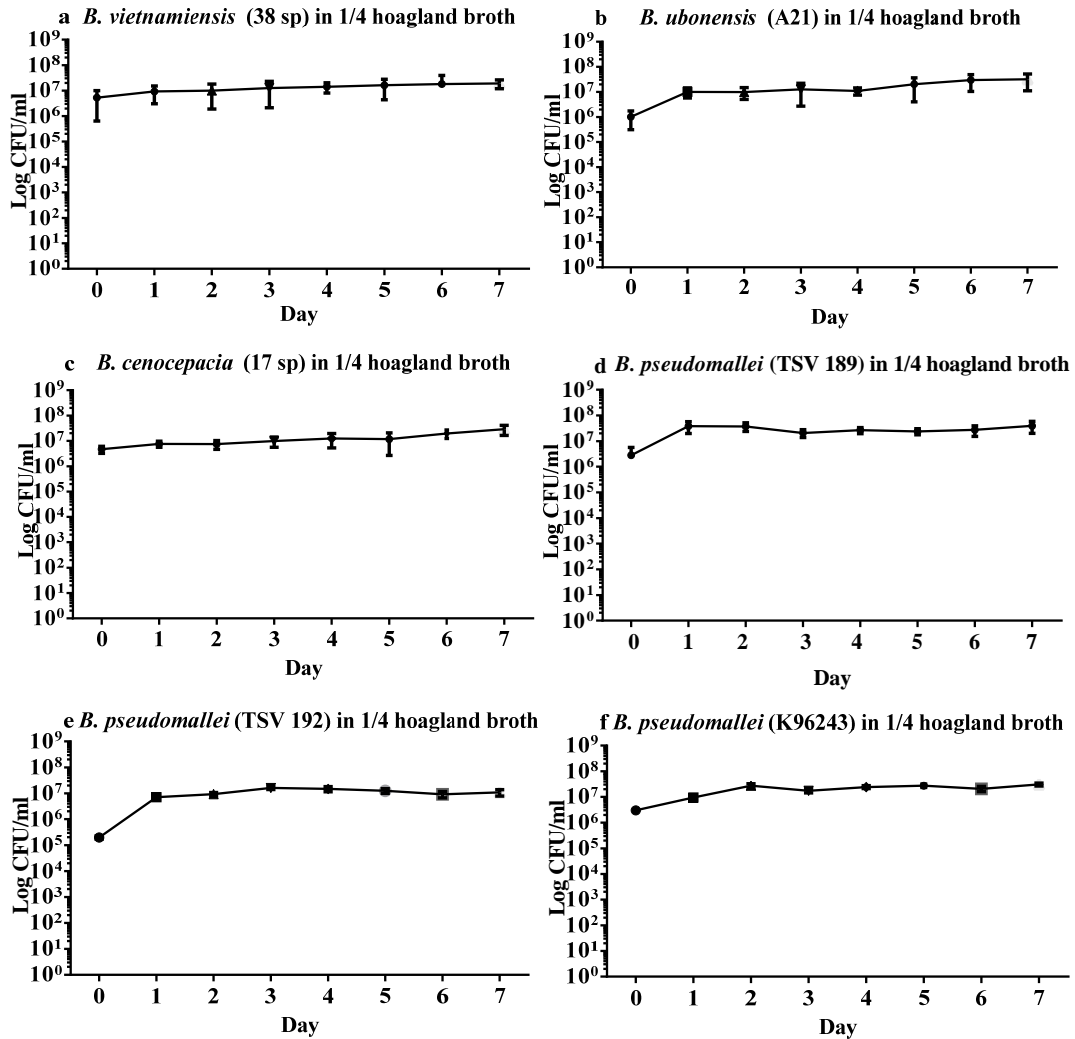
6 **METHODS**

7 Bacteria were cultured overnight in 10ml LB broth at either 37 °C (*Burkholderia*  
8 *pseudomallei* or 30 °C (other species). Cultures were centrifuged at 3000g for 15 min and the  
9 supernatant discharged. To wash the pellet, 10ml of 0.85% NaCl was added to the pellet. The  
10 pellet was resuspended and the solution centrifuged at 3000g for 15 min. The supernatant  
11 was discharged and the process repeated three times. Finally, the pellet was made up in  
12 0.85% NaCl to 10ml (about 10<sup>8</sup> CFU/ml). One hundred µl of bacterial suspension was added  
13 to 9.9 ml of ¼ strength Hoagland solution and incubated at 30 °C. Twenty ul samples were  
14 removed each day from day zero to seven. Bacteria in samples were counted via plate counts.  
15 Statistical significance was calculated using an ANOVA with Games-Howell (p=0.05) from  
16 day one to seven. Games-Howell was used in this experiment due to heterogeneity of  
17 variance.

18

19 **RESULTS**

20 While initial loads varied at day zero between 10<sup>5</sup> cfu/ml and just under 10<sup>7</sup> cfu/ml, this had  
21 stabilised by day one. Bacterial numbers were then stable between day one and day seven  
22 (supplementary figure 1), with no significant difference between this different species and  
23 strains of bacteria (ANOVA p values >0.05, Games Howell post hoc test)



24

25 **Supplementary Figure 1:** Survival data (mean  $\pm$  95% CI) obtained in 1/4 strength Hoagland  
 26 solution for near neighbour species *B. vietnamiensis* 38sp (a), *B. ubonensis* A21 (b), *B.*  
 27 *cenocepacia* 17sp (c) and *B. pseudomallei* (TSV189 (d), TSV192 (e), and K96243 (f)).

28

29 **DISCUSSION**

30 Hoaglands broth contains a range of carbon sources (1). Initial growth of bacteria may be due  
 31 to energy sources already present in the bacteria as well as use of some of these carbon  
 32 sources. Survival kinetics for all *Burkholderia* species and strains in Hoaglands broth were

33 similar. This indicated that the effects of Hoagland agar on the bacteria can be excluded as a  
34 cause of experimental variation.

35

36 **REFERENCES**

- 37 1. **Hoagland DR, Arnon DI.** 1950. The water-culture method for growing plants without  
38 soil. Circular: California Agricultural Experiment Station **347**:1-32.