

Developing a novel assay to study biofilm formation in *Galleria mellonella*.

Acinetobacter baumannii is a Gram-negative opportunistic pathogen associated with nosocomial infections such as pneumonia, meningitis, and wound and soft-tissue infections (Zeighami *et al.*, 2019). The pathogen is able to form biofilms on both biotic and abiotic surfaces and the *A. baumannii* isolates specifically have a much higher biofilm formation rate (80-91%) than other *Acinetobacter* species (5-24%) (Thummeepak *et al.*, 2016; Sung, 2018). The bacteria inside these biofilms can be up to 1000x more resistant to higher concentrations of antibiotics than when they're in their planktonic mode, making the infections more difficult to treat (Steward, 2014).

Current *in vivo* biofilm formation assays are carried out in larger organisms such as mice and rats. This study tried to use *Galleria mellonella* as a substitute for these organisms. *Galleria* are wax moths that have a similar innate immune system to that of vertebrate models, however they are not subject to Home Office Regulations, which reduces the ethical issues associated with the assays.

Method

The study consisted of injecting specific concentrations of different *A. baumannii* strains (wild-type, a high biofilm former, and a low biofilm former) into the *Galleria* along with beads (fig. 1). The *Galleria* were then incubated overnight allowing the bacteria to grow within them and hopefully attach to the beads. The following day the beads would be extracted from the *Galleria* (fig. 2) and any bacteria attached to them would be removed, plated out and incubated overnight. Any colonies formed the next day were counted and compared to the controls to see if there was any bacterial attachment.



Fig. 1: Injecting *Galleria mellonella* with beads and bacteria.



Fig. 2: Extracting beads from *Galleria mellonella*

Results

The results obtained towards the beginning of the study didn't really show much of a notable difference in the levels of attachment, but as time went on the method had been practiced and refined more the results started to show differing levels of attachment. Fig. 3 shows the results for the final test carried out and it can be seen that Mutant 1 has similar levels of biofilm formation to the wild-type, while the Mutant 2 strain has much higher levels of biofilm formation. This is interesting to see considering that in previous *in vitro* tests Mutant 1 was shown to be a consistently high biofilm former

while Mutant 2 was a consistently low biofilm former. This suggests that certain strains of *A. baumannii* may grow differently when *in vivo* compare to *in vitro*, meaning that there may be a substance within the *G. mellonella* that inhibits biofilm formation and could therefore make *A. baumannii* infections easier to treat. This study also shows that, one the method becomes a bit more refined, *Galleria mellonella* can indeed be used to as a model organism to study biofilm formation and can therefore replace larger organisms such as mice and rats in the future.

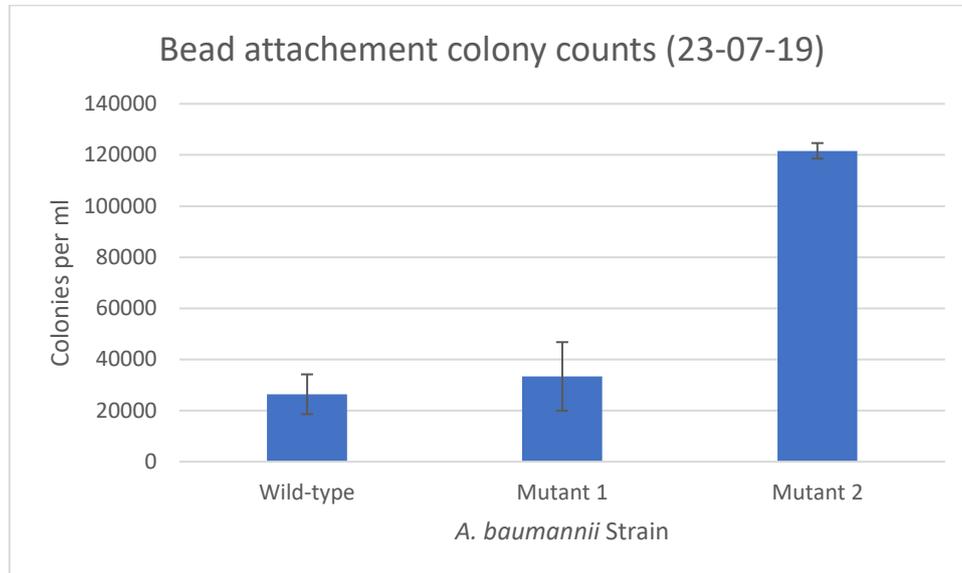


Fig. 3: Graph showing the number of colonies per ml attached to the beads for each strain of *A. baumannii* tested.

References

- Stewart, P.S. (2015) 'Antimicrobial Tolerance in Biofilms', *Microbiology spectrum*, 3(3). doi: 10.1128/microbiolspec.MB-0010-2014.
- Sung, J.Y. (2018) 'Molecular Characterization and Antimicrobial Susceptibility of Biofilm-forming *Acinetobacter baumannii* Clinical Isolates from Daejeon, Korea', *The Korean Journal of Clinical Laboratory Science*, 50(2), pp. 100-109. doi: 10.15324/kjcls.2018.50.2.100.
- Thummeepak, R., Kongthai, P., Leungtongkam, U. and Sitthisak, S. (2016) 'Distribution of virulence genes involved in biofilm formation in multi-drug resistant *Acinetobacter baumannii* clinical isolates', *International microbiology: the official journal of the Spanish Society for Microbiology*, 19(2), pp. 121-129. doi: 10.2436/20.1501.01.270.
- Zeighami, H., Valadkhani, F., Shapouri, R., Samadi, E. and Haghi, F. (2019) 'Virulence characteristics of multidrug resistant biofilm forming *Acinetobacter baumannii* isolated from intensive care unit patients', *BMC Infectious Diseases*, 19(1), pp. 629. doi: 10.1186/s12879-019-4272-0.