Viewing *Euprymna berryi* as a Host in Testing *Vibrio fischeri*

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Introduction

Background Information

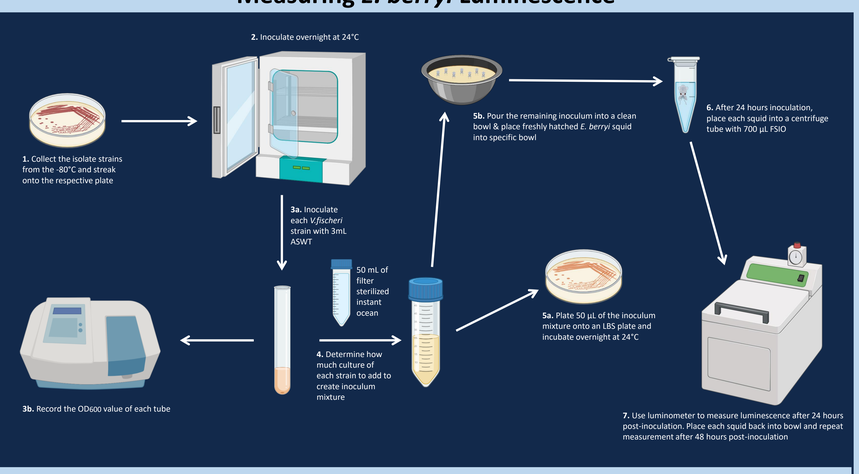
- *Euprymna berryi* are cephalopods found in the Indo-Pacific Isolands[1] and are the first species to successfully receive gene editing through CRISPR-Cas9 that deactivated genes for two pigmentation enzymes[2]
- The creation of the "albino squids" allows for the optical access of the nervous system in a living cephalopod
- This characteristic is bred through multiple generations, making them ideal for testing the luminescent marine bacteria, *Vibrio fischeri*, in the light organ[1]
- Similar to a past study observing Vibrio-squid symbiosis of *V. fischeri* and *Euprymna scolopes* squid,[3] the symbiosis of *Vibrio fischeri* strains and *E. berryi* were observed to view what kind of strains colonize the cephalopod host, as well as their efficiency

Objectives

- Use luminescence to determine if the *V.fischeri* isolate strains, ES401, ANS2100, WEB2, WEB9, and HI2, colonize *E. berryi*
- Figure out the colonization and inoculation levels of the *V. fischeri* strains
- Determine if *E. berryi* squid are sufficient host animals when testing *V. fischeri*.

Methods

Measuring *E. berryi* Luminescence



Determining Colonization Levels

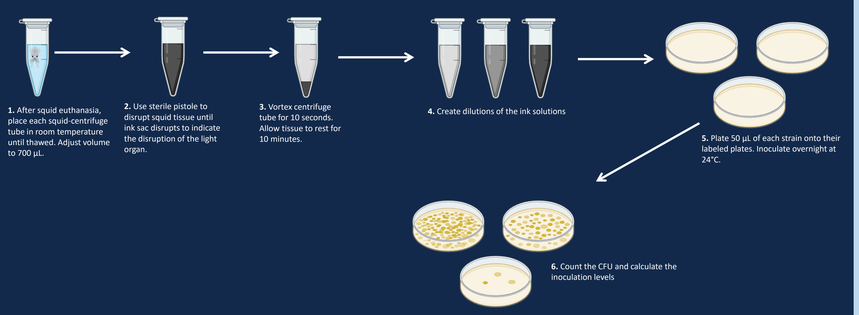


Image 1. Image *of Euprymna berryi* squid by Morgan N. Pavelsky.

Results

Luminescence Levels

A		Vibrio Fischeri Strain (10.10.2023)						
Squid #	Apo	WEB2	WEB4	WEB5	WEB9	HI2	ES401	ES114
1	76	219,852	95	90	10,257	89,950	103,094	17,525
2	73	1,046,409	91	78	112,181	1,965	21,021	28,941
3	171	488,093	112	105	237,008	152,953	5,840	10,588
4	122	498,625	123	92	1,659	181,807	14,458	33,853
5		118,221	108	98	13,390	152,850	11,756	30,871
6		926,782	130	91	261,407	120,993		
7		9,440	91	89	451,703	1,332		

B		Vibrio Fischeri Strain (10.11.2023)						
Squid #	Apo	WEB2	WEB4	WEB5	WEB9	H12	ES401	ES114
1	90	11,404	118	115	24,974	24,497	1,919	7,083
2	35,603	16,781	127	97	216,959	61,193	33,441	24,221
3	107	16,104	113	93	439,523	4,735	8,968	721
4	393	22,665	103	115	282,293	13,284	125	10,095
5		17,104	112	125	105,497	62,668	5,002	23,855
6		10,075	88	80	31,690	1,695		
7		57,095	114	99	495,830	3,638		

Table 1A and Table 1B. Measured *Vibrio Fischeri* Luminescence Values at 24 Hours Post-Inoculation and 48 Hours Post-Inoculation. After 24 hours post-inoculation, the luminescence value of the colonized *E. berryi* squid were recorded on Table 1A, and the 48-hour post-inoculation luminescence values of the *E. berryi* squid were on Table 1B. Luminescence values of 1,000 or higher are classified as colonized. The Table 1A reveals that WEB4 and WEB5 did not properly colonize the *E. berryi* squid as their values matched the apo-symbiotic squid, which served as the controlled variable in the experiment.

CFU/Squid Count

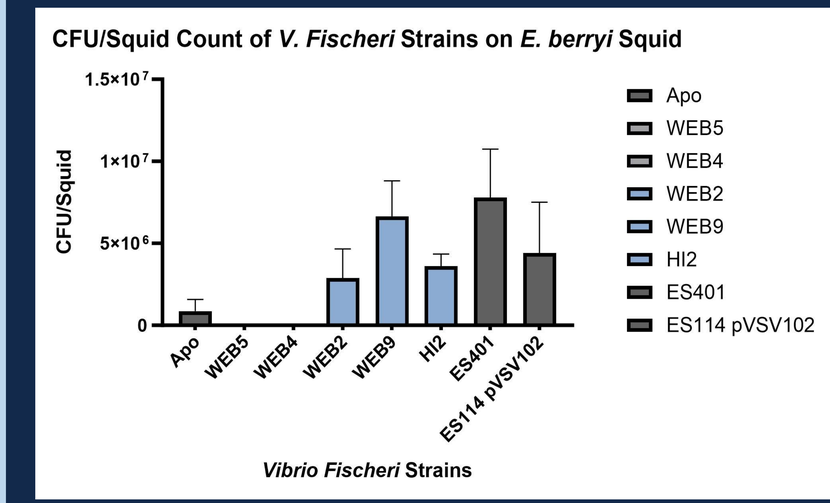


Figure 1. CFU/Squid Count of *Vibrio Fischeri* Strains *on E. berryi* Squid. The CFU/Squid count is represented by Figure 1 that determines the colonization levels of each strain and the apos. The CFU/Squid axis ranges from 0 to 5×10^7cells. This figure reveals all strains has high colonization levels, except for WEB4 and WEB5 that showed little-to-no levels. This further shows why WEB4 and WEB5 are not good strains to use when testing for Vibrio-squid symbiosis using *E. berryi* as hosts, while WEB2, WEB9, and HI2 are sufficient. For statistical analysis, two values of the apo squid were deleted from the data set due to high uncertainty caused by human error of the transferring squid step.

Conclusion

Reflections

- The luminescence and CFU/Squid count were consistent with each other
- Determined that *E.berryi* is a good host when analyzing the symbiotic Squid-Vibrio relationship

Future Directions

- Create co-incubation assays to determine which *E. berryi* Vibrio strains express the type VI secretion system (T6SS) gene
- For strains that express the T6SS gene, use conjugation to successfully create a mutant gene that no longer has the gene

Acknowledgements

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