

# Isolation & utilization of probiotics to manage epizootic shell disease in American lobster, *Homarus americanus*

Melissa Hoffman\*, Kathleen Castro, Grace Underwood, Hilary Ranson, Barbara Somers, Mitch Hatzpietro, David Rowley, David R. Nelson, Marta Gomez-Chiarri  
 \*Department of Fisheries, Animal, and Veterinary Sciences, University of Rhode Island, Kingston, RI (melissahoffman@uri.edu)

## Introduction

### Epizootic shell disease (ESD)

- Characterized by melanized lesions in cuticle.
- Negatively impacts molting, growth, survival, and reproduction → declining populations.
- Impacts 30% of lobsters in Narragansett Bay, RI.
- ESD mostly limited to southern New England.

### Probiotic use in shellfish

- Probiotics have been successful in protecting many aquacultured organisms from disease.
- Probiotics can have a direct antibiotic effect, compete for space with pathogenic bacteria, and/or modulate immune response of the host.

**Goal:** Identify candidate bacterial probiotics, previously isolated from lobsters, that could slow down or stop the progression of ESD in lobsters.

## Methods

### Objective 1: Screen bacterial strains on lobster post-larvae (PL's)

- PLs treated 2x with 7 bacterial treatments over 3 weeks.
- 12 PLs per treatment, one control group.
- Challenged with candidate pathogen *Thalassobius* spp., a bacterial strain thought to play a role in ESD infection.
- Measured mortality and incidence of shell disease.



### Objective 2: Test candidate probiotics on adult lobsters with ESD

- Treated 2x a week with 3 probiotics over 3 months.
- 10 lobsters per treatment, one control group.
- Pictures taken before/after and after any molt.

- Measured growth, molting, and changes in ESD lesions.
- Extracted hemolymph for future qPCR gene expression analysis.



## Results

### Objective 1: Screen bacterial strains on lobster post-larvae

Table 1. List of isolated bacteria from lobsters in Narragansett Bay used as probiotic candidates. Average zone of Inhibition (ZOI) measured in millimeters from the edge of colony against putative lobster pathogens *Thalassobius* spp. and *Aquimarin* sp. were tested, and average biofilm was measured using Optical Density 580nm (OD 580) staining (Zhao et al. 2016).

Isolate ID	Identification by 16s rRNA Sequencing	ZOI (mm) against <i>Thalassobius</i> spp.	ZOI (mm) against <i>Aquimarin</i> sp.	Biofilm Measurement (OD 580)
P21	<i>Pseudoalteromonas</i> sp.	1.16	0	2.18
P14	<i>Pseudoalteromonas</i> sp.	2.50	0	3.24
B	<i>Bacillus</i> sp.	2.00	4.33	2.88
P22	<i>Pseudoalteromonas</i> sp.	1.83	0	3.71
L210	<i>Loktanella maritima</i>	2.00	0	3.71
L211	<i>Loktanella maritima</i>	1.66	0	3.71
S4	<i>Phaeobacter inhibens</i>	1.00	1.40	3.70

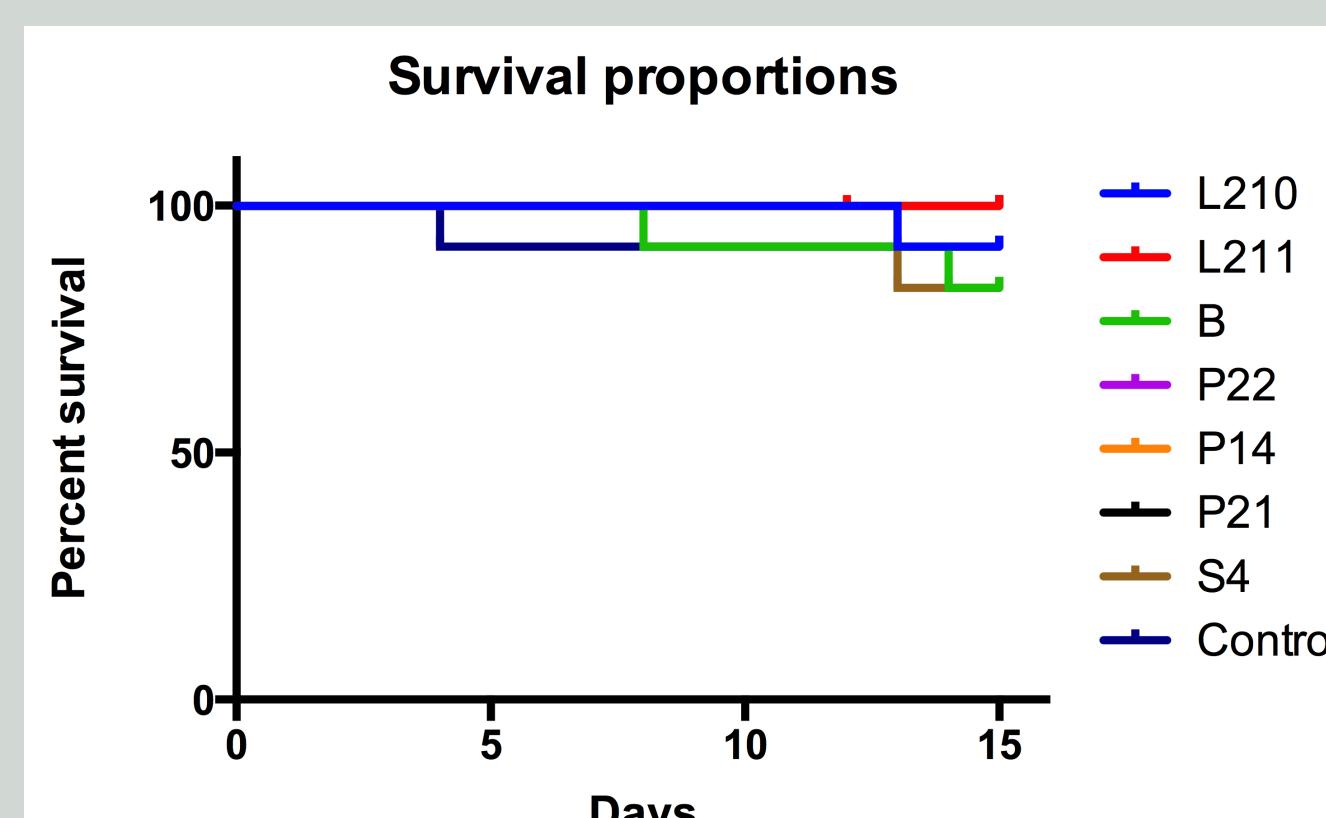


Figure 1. Percent survival of PL's over 15 days. At time 0, probiotics were administered to PL's and one day later they were challenged with *Thalassobius* spp. Mortality was minimal and no significant differences in survival between treatments were observed (Log-rank test  $p=0.27$ , Prism 6).

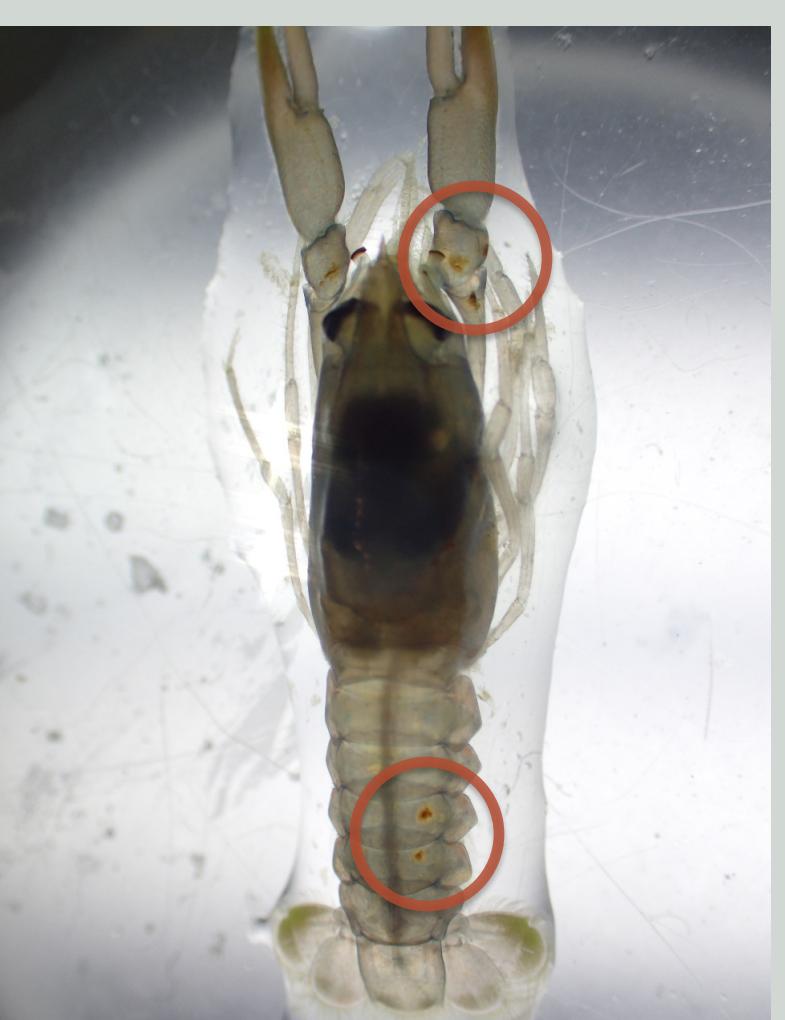


Figure 2. Photo of a PL used in the experiment exhibiting lesions on the tail and claw (circled).

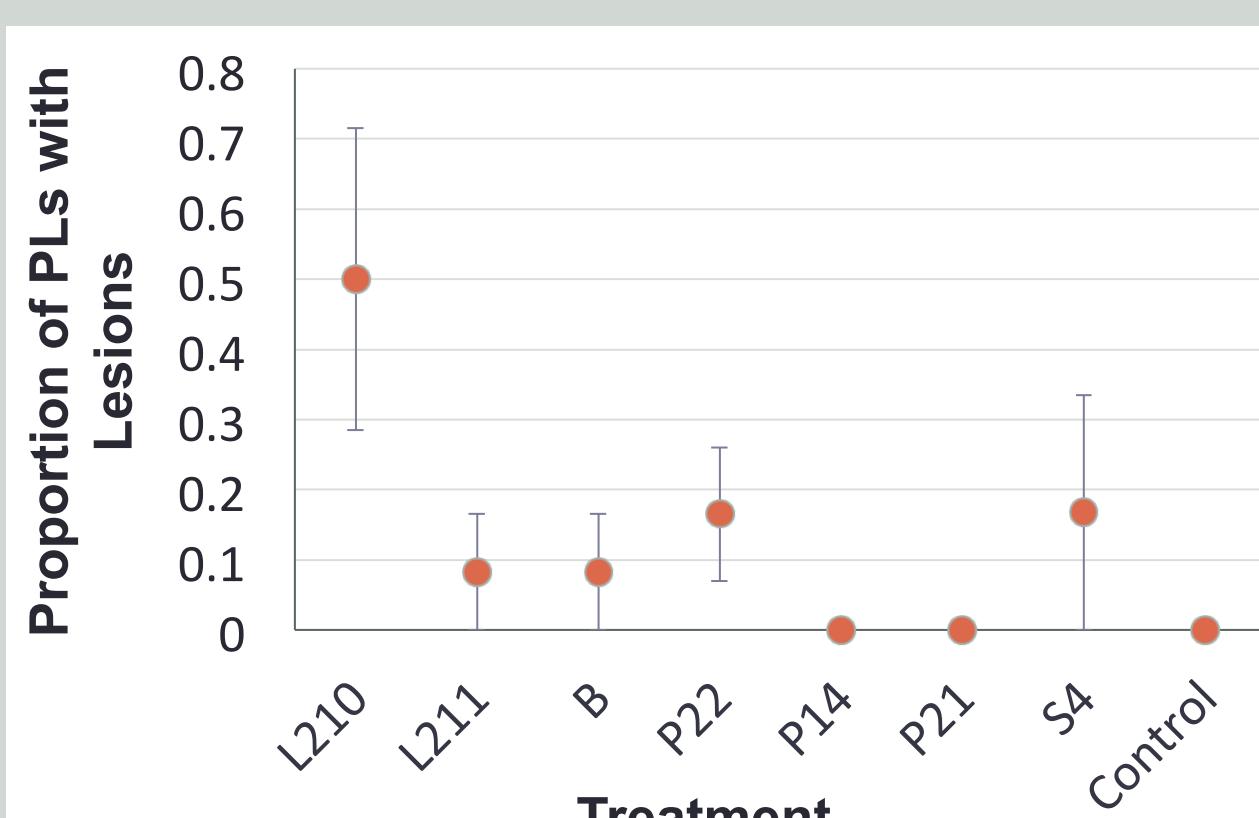


Figure 3. Mean proportion of PLs in each treatment with lesions after 15 days. Averages were taken from the proportion of lobsters in each treatment (3 per beaker, 4 replicates) with lesions. Although no statistical differences were observed between groups (chi-square  $p=0.43$ ), L210 showed the highest proportion of shell disease.

### Objective 2: Test candidate probiotics on adult lobsters with ESD

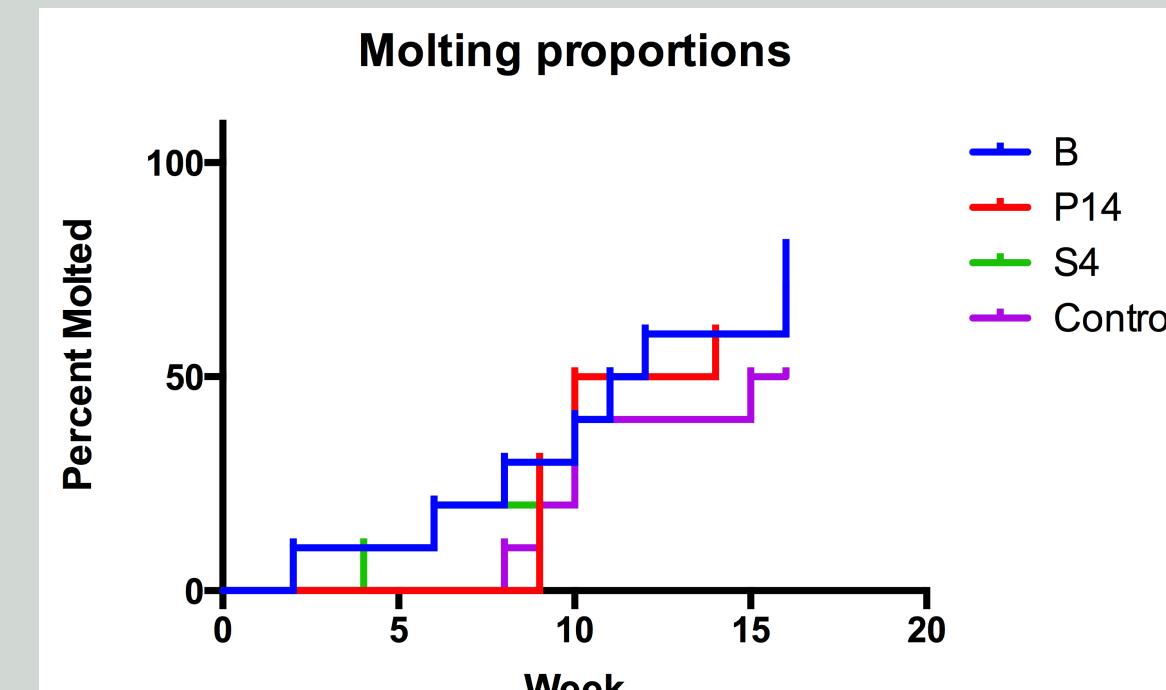


Figure 4. Percent of individuals that molted over the 16 weeks of treatment. No statistical differences in molting were observed between treatments (Log-rank test  $p=0.69$ , Prism 6).

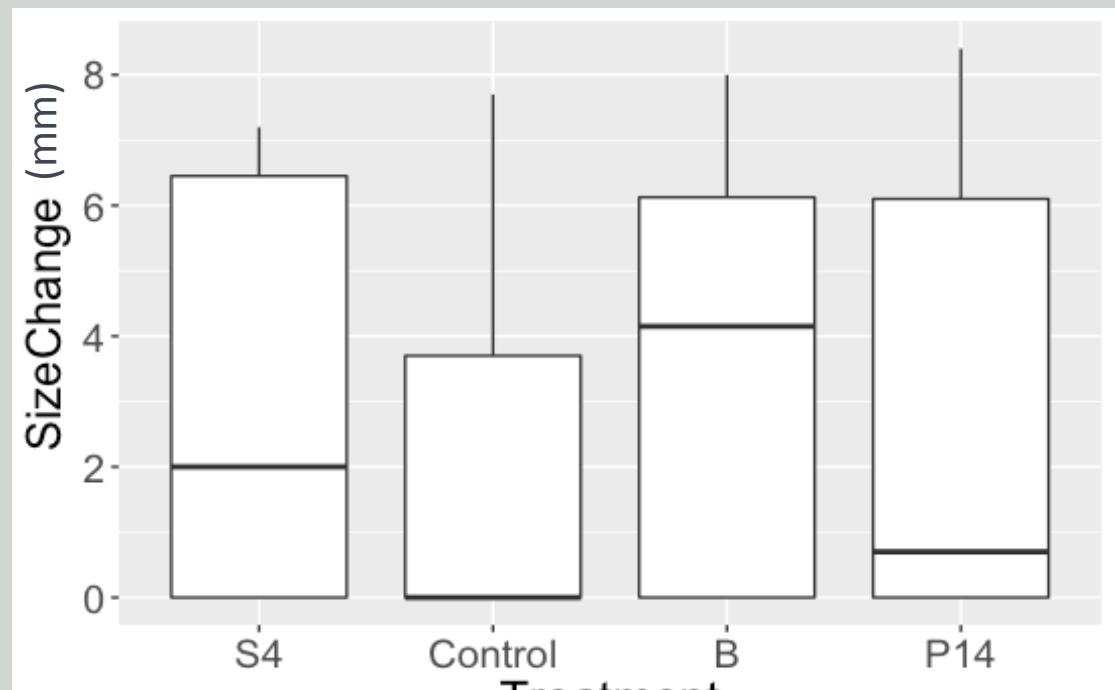


Figure 5. Average change in size of lobsters for each treatment. There were no statistical differences in growth between treatments (One-way ANOVA  $p=0.82$ , R Studio).

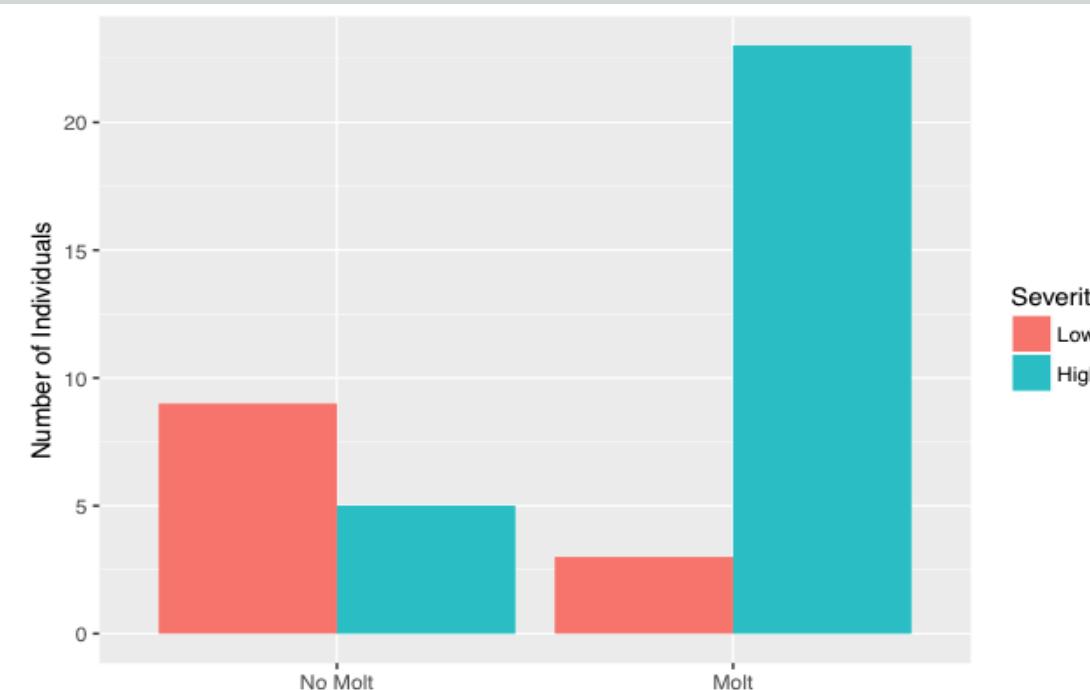


Figure 6. Individuals that molted in relation to severity of ESD. "High Severity" indicates lesions covered >50% of carapace. There was a significant relationship between severity of ESD and molting (Chi-square test  $p=0.0016$ ), but not between molting and treatment (not shown).



Figure 7. An example of photographs taken of one lobster in the S4 treatment at the beginning of experiment, when molting occurred, and at the end of the experiment. All lobsters that molted did not exhibit any signs of shell disease after the molt, including lobsters that molted towards the beginning of the experiment.

## Discussion

### Objective 1: Screen bacterial strains on larvae

- There was no significant differences in molting (not shown) or survival between treatments.
- Though there was no significant difference between treatments, the presence of lesions in almost all PLs indicates it could be a good model system for screening probiotic candidates.
- Based on results from Table 1 and *in vivo* PL trial, probiotics were chosen for the adult experiment.

### Objective 2: Test candidate probiotics on adult lobsters with ESD

- There were no significant differences observed in molting or growth between treatments.
- High incidence of molting was attributed to disease severity rather than treatment.
- None of the lobsters got ESD again after molting.
- Many lobsters could have been too advanced in ESD stage for visual improvements in carapace from probiotic treatments.

## Future Work

- Repeat PL experiments to determine if lesions are naturally occurring, or if they are result of probiotic treatments.
- Conduct qPCR on hemolymph samples from the adult lobster trial to investigate expression of immune- and molting-related genes: Homome ALF-1, Hoa-crustin, EcR-receptor

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