



# Differential transcriptional immune response in the reef building coral, *Acropora millepora*, to ecologically relevant bacteria

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## Abstract

**Background:** Disease outbreaks on coral reefs are increasing rapidly. How the coral immune system responds to disease and bacterial challenge is poorly understood. *Vibrio coralliilyticus* and *Alteromonas* sp. are two known bacterial pathogens of corals such as *Acropora millepora*. Here we explored the immunological response of the reef building coral *A. millepora* to both bacteria.

**Methods:** *A. millepora* coral fragments were challenged for 48 hours with *V. coralliilyticus* or *Alteromonas* sp. at a concentration of 10<sup>6</sup> CFU/ml. Total RNA was extracted from samples collected at 6 and 24h and reverse transcribed to examine gene expression by quantitative real time PCR (qPCR) of 3 putative innate immune genes: complement component 3 (C3), C-type lectin, and heat shock protein 70 (Hsp70). Denaturing gradient gel electrophoresis (DGGE) was run on the 24h samples to study changes in the coral-associated microbial community.

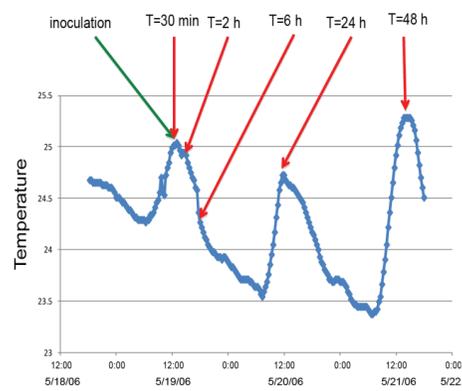
**Results:** After 48h, coral fragments challenged with *V. coralliilyticus* appeared healthy while fragments challenged with *Alteromonas* sp. showed signs of bleaching and tissue lesions. Correspondence analysis of the microbial community showed that the bacterial community associated with the coral fragments inoculated with *Alteromonas* sp. were different from controls and *V. coralliilyticus* inoculated colonies. QPCR data showed a significant increase in gene expression of *c3-like* and *hsp70* in *Alteromonas* sp. inoculated corals at 24h. *Hsp70* showed a significant increase in the *V. coralliilyticus* treatment at 24h. *C-type lectin* did not show a response due to treatment.

**Conclusion:** *V. coralliilyticus* did not cause visual signs of disease but an increase in *hsp70* expression indicates that the corals were stressed. However, treatment with *Alteromonas* sp. did cause signs of disease. The increase of expression of *c3-like* and *hsp70* in these infected corals indicates their involvement in the immune response of *A. millepora*. A shift in the bacterial community associated with the diseased corals was detected suggesting that an increase in abundance of one or more opportunistic bacteria could have been the cause of the disease rather than the inoculated bacterium. Further investigation is needed in this regard.



## Methods

**Figure 1:** The tank set up for the experiment. *A. millepora* nubbins from colony A were placed into tanks 1-3 and challenged with 10<sup>6</sup> CFU/ml of *V. coralliilyticus* while the controls were placed into tanks 4-6. Nubbins from colony B were challenged with *Alteromonas* sp. at a concentration of 10<sup>6</sup> CFU/ml in tanks 7-9. The controls from this colony were also placed into tanks 4-6.



**Figure 2:** Sampling times for the experiment as well as the temperature profile. 6 and 24h samples were assessed by QPCR. 24h samples were assessed by DGGE.

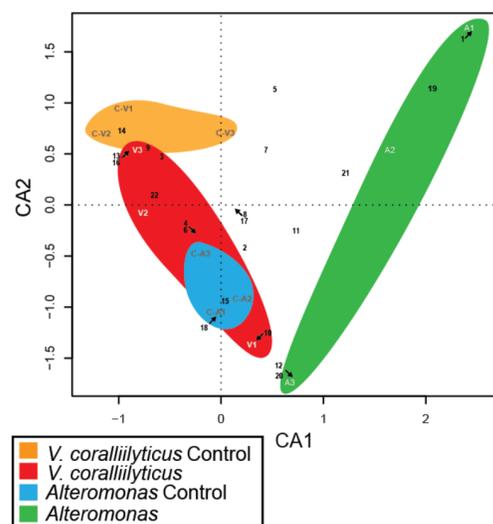
QPCR was performed using the Roto Gene SYBR Green PCR master mix (Qiagen, Valencia, CA). Primer concentration of 1 μM was used for *c3-like*, *hsp70*, *rpl12*, and *actin*. A 0.5 μM primer concentration was used for *c-type lectin*. Primer sequences for the 3 genes of interest are found below.

Gene	Primers (5' to 3')
<i>c3-like</i>	For: GTGAAGGTGGAACCAGAGGA Rev: GAACCGGAAGTGATTGTCGT
<i>c-type lectin</i>	For: CAGTCTGGATCGGACTCAT Rev: CATGTCCAGTGGTTGTACGC
<i>hsp70</i>	For: GAGCCCTCAGTAACCAGCAC Rev: CATTGTGGAGCGAAAAAGTT

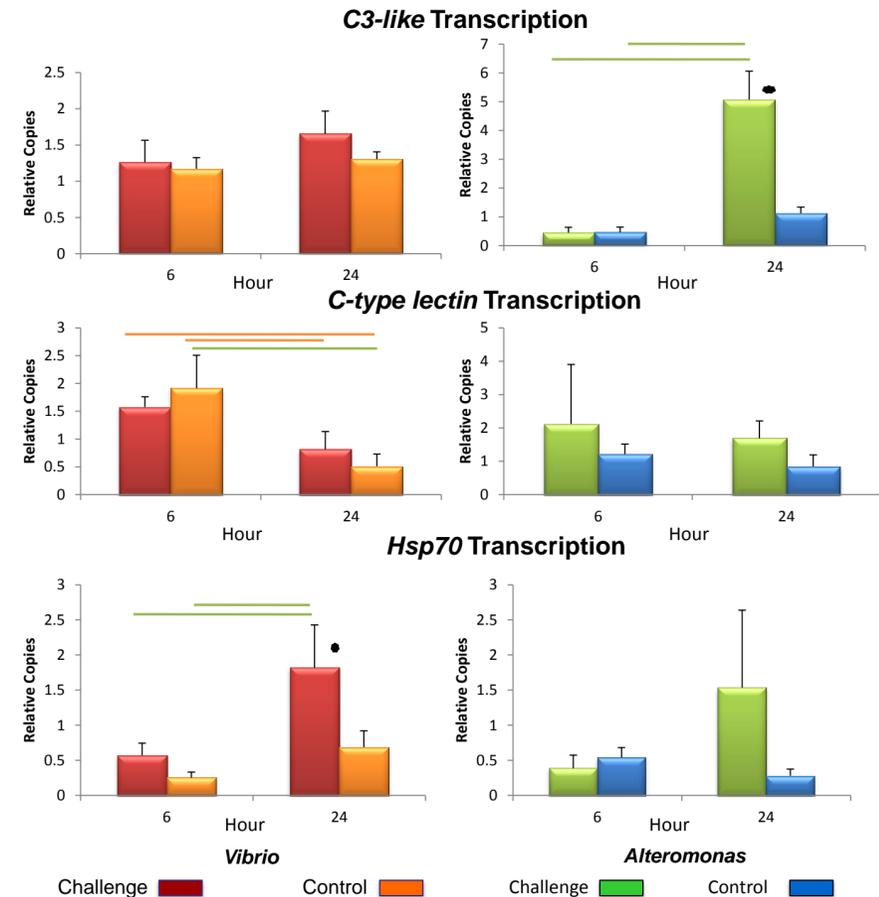
## Results



**Figure 3:** Photographs of *A. millepora* nubbins at the conclusion of the 48 hour experiment. *V. coralliilyticus* and control nubbins appear healthy while *Alteromonas* sp. inoculated nubbins show signs of tissue lesions (white color).



**Figure 4:** Correspondence analysis (CA) of the bacterial 16S rDNA-DGGE banding pattern of the microbial community associated with the control and challenged coral nubbins 24h post inoculation. CA1 accounts for 26.9% of the variation and CA2 accounts for 20.8% of the variation. CA1 separates *Alteromonas* sp. inoculated nubbins from both sets of controls as well as *V. coralliilyticus* inoculated nubbins. Numbers indicate gel bands.



**Figure 5:** Relative expression of *c3-like*, *c-type lectin*, and *hsp70* in coral nubbins challenged with *V. coralliilyticus* and *Alteromonas* sp. at 6 and 24h post inoculation. Lines above bars indicate significance at  $p < 0.01$  (green),  $p < 0.05$  (orange), (\*) between treatment and control. Error bars indicate standard deviation. *Alteromonas* sp. inoculated nubbins show a significant increase in expression for *c3-like* and *hsp70*. *V. coralliilyticus* inoculated nubbins show an increase in expression for *hsp70*.

## Conclusions

- At ambient seawater temperatures and 10<sup>6</sup> CFU/ml, *V. coralliilyticus* does not appear to cause disease while *Alteromonas* sp. does.
- Increases in the transcriptional response of *hsp70* and *c3-like* in the *Alteromonas* sp. inoculated nubbins would indicate that a potential immune response was elicited in *A. millepora*.
- Increase in expression of *hsp70* in *V. coralliilyticus* inoculated nubbins would indicate that these nubbins were under stress.
- Correspondence analysis indicates that a shift in the bacterial community occurred in the *Alteromonas* sp. inoculated nubbins.
- Disease signs in *A. millepora* may have been caused by an opportunistic group of bacteria as a result of the microbial community shift rather than caused directly by *Alteromonas* sp.

## Acknowledgements

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