

Interactive comment on “Finding immune gene expression differences induced by marine bacterial pathogens in the deep-sea hydrothermal vent mussel *Bathymodiolus azoricus*” by E. Martins et al.

Anonymous Referee #1

Received and published: 30 April 2013

General Comments: The authors analyzed gene expression in the hydrothermal vent mussel *Bathymodiolus azoricus* in response to challenge by different cultured bacteria. The results of this study advance our knowledge of innate immunity in hydrothermal vent organisms.

That stated, I feel this manuscript needs a major revision for the results and discussion section. I would like to see more of a discussion of how the genes analyzed may be involved with responding to pathogens by *B. azoricus*. The results and discussion section is mainly results and more discussion would greatly improve the manuscript. How

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do the authors' results compare to similar studies conducted with other bivalves, for example estuarine oysters (for which there is a rich literature on pathogen response)? For each of these innate immunity genes there is a literature on how these genes and gene products interact with pathogens. There are a few papers on innate immunity in hydrothermal vent organisms that should be cited or discussed as well (*Alvinella pompejana*; Gagniere et al., 2010) and *Ridgeia piscesae*; (Nyholm et al., 2012). It would also be useful to the reader if the authors could discuss this present work in the context of their previous work on innate immunity in *B. azoricus*.

Specific Comments: Because the mussels were not kept under in situ pressure, this additional stress may influence gene expression. Do the authors have any evidence that *B. azoricus* can be maintained and function “normally” under ambient pressure conditions?

Why did the authors choose *Flavobacterium* and what do they make of the fact that the greatest changes in gene expression (for the lectin and lysozyme) were in response to this bacterium at 12h? Is there any evidence of *Flavobacterium* acting as a pathogen?

Please provide the strain names for the bacteria used. Have any of these bacteria been described at hydrothermal vents?

Fig. 8. I could not tell any differences between the gels highlighted by the rectangles in the 24 h treatment. What exactly are the main protein differences? The authors need to be more explicit here. There is also an area in brackets that is not defined in the figure legend. Related to this: p. 2687. The authors report that actin was identified as being reduced between 12 h and 24 h but there is no discussion as to the significance of this. There is a rich literature on how pathogens influence host actin and this should be included in the discussion.

p. 2684, line 27-28: there are examples of other molluscs discriminating between vibrios (ie., in squid *E. scolopes* and *V. fischeri* and non symbiotic vibrios)

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p. 2689: I would like to see more discussion about the specific dynamics that the authors refer to. Also, adding a couple of sentences to address how the endosymbionts might factor into the host immune response would be useful. These are located in the gills so presumably the host cells are also being exposed to MAMPS/PAMPS on a continual basis.

Appendix A: (Protein sequence) please add more information here so the reader understands what protein is being showed.

Technical Comments:

p. 2676 line 13; irrelevant not best adjective here line 13; delete times p. 2677, line 8; biomass to members? Line 20; pathogen-associated molecular patterns (PAMPS) although still used is not as accurate as microbe-associated molecular patterns (MAMPS) since these compounds are not unique to pathogens Line 24: Invertebrate and molluscan immune response p. 2678, line 6: *Vibrio* spp are a major cause of disease in the marine environment line 13 organisms to bivalves? p. 2681 line 10: pairs to pair p. 2682 line 8 : considered to analyzed? Line 17: 13,200 Line 19: period at end of sentence Line 22: were loaded to was loaded Line 26: and with gentle agitation p. 2683, line 9: Below seawater gene expression levels. . . (awkward consider rephrasing) line 27: compared to the control(seawater).

p. 2686, lines 8-10: This sentence is awkward (consider rephrasing) line 25: as for the control. . . to . . .as the control, p. 2687, line 12: delete "the" in front of epidermal p. 2688 line 15: This supports the possibility line 2688: lines 23-24. We conclude from this study that *B. azoricus* mussels were under stress conditions. . ." (I would be specific here and state that your data suggest that they were mounting an immune response).

Interactive comment on Biogeosciences Discuss., 10, 2675, 2013.

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