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Whole transcriptome analysis reveals changes in expression of immune-related genes during and after bleaching in a reef-building coral

Jorge H. Pinzón, Bishoy Kamel, Colleen A. Burge, C. Drew Harvell, Mónica Medina, Ernesto Weil and Laura D. Mydlarz

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Original submission: 7 August 2014
Revised submission: 2 November 2014
Final acceptance: 4 March 2015

Note: Reports are unedited and appear as submitted by the referee. The review history appears in chronological order.

Review History

RSOS-140214.R0 (Original submission)

Review form: Reviewer 1 (Lory Santiago)

Is the manuscript scientifically sound in its present form?
Yes

Are the interpretations and conclusions justified by the results?
Yes

Is the language acceptable?
Yes

Is it clear how to access all supporting data?
Yes, I was able to access the website and download and open the files.

Do you have any ethical concerns with this paper?
No

Have you any concerns about statistical analyses in this paper?
I do not feel qualified to assess the statistics

Recommendation?
Accept with minor revision (please list in comments)

Comments to the Author(s)

- Abstract and introduction are well written. They provide the necessary background to put the presented experiments into context. Their experimental subjects are appropriate to test their hypothesis since these are naturally bleached O. faveolata colonies compared to non-bleached colonies during the same environment and same time period. The value of this project lies in the fact that the results that they got are very close to what happens in nature so this information can be directly used to design conservation strategies and management plans.
- One question I had right from the start was related to the authors' comments on corals of the same species but living in warmer vs. colder environments and how the warmer colonies are more resilient than the colder ones. Is this something that they plan to test in the future since they spend a paragraph on this topic? This is not addressed again in the rest of the manuscript. I would also be interested to if in the long-term, these previously bleached but now recovered corals are more susceptible to disease or bleaching; does a bleaching renders them immunologically suppressed for as long as the colony is alive? Would like to see this point discussed in the discussion.
- Typo: Page 6, line 128 should say "manufacturer's" instead of manufacture's
- Methodology is very clear and also well written. RNA-Seq is highly technical but authors did a good on presenting essential information in an easy to understand manner.
- Page 9, lines 180-183: What is the purpose of the additional samples collected in Dec. 2010 and August 2011? I didn't find them used in the rest of the experiment but maybe I missed it.
- Page 11, paragraph starting in line 235: To clarify, do authors mean that during the sampling period the transcriptome of Symbiodinium of bleached and unbleached did not change the whole time yet the species associated while the bleached corals transcriptome did change? Or that the changes were the same as those seen in the metatranscriptome and that the metatranscriptome did change as discussed in previous paragraph?
- Page 13 line 267: annotated genes seem to return to normal expression levels a year after the bleaching event (no difference between bleach vs. unbleached)? Or is it that what was differentially expressed in Nov. 2010, returned to normal in March 2011, but were again differentially expressed almost a year later? This paragraph is a bit confusing. Also, if I am understanding the data correctly, I'm wondering if the return to differentially expressed genes a year later predates a future bleaching event or the onset of disease after October 2011. I'm sure the authors are continuing to monitor these colonies and hopefully they'll follow up on their status. Is it possible to comment on the health of those colonies (at least visually) for this manuscript? If not, this wouldn't disqualify this manuscript for publication; I'm just curious.
- Page 15 paragraph on line 315. When authors refer to the holobiont expression profile I am assuming that it is the same as the metatranscriptome? But expression of bleached colonies grouped Nov 10 and Oct 11 together although not March 11, yet everything else part of the holobiont (Symbiodinium and other eukaryotes) grouped with the unbleached metatranscriptome or the metranscriptome as a whole (bleached + unbleached)? Please clarify this paragraph a little better.
- The Mydlarz lab specializes in work at the protein level so I am wondering if the authors plan to look at the expression of some of these genes at the protein level to see if they correlate. There's a lot of regulation that happens post-translationally and I am wondering if they plan to look at this.
- Overall this was an excellent paper and I enjoyed reading it very much.

Review form: Reviewer 2

Is the manuscript scientifically sound in its present form?
No

Are the interpretations and conclusions justified by the results?
Yes

Is the language acceptable?
Yes

Is it clear how to access all supporting data?
No. Analyses make use of considerable unpublished genomic resources (see lines 147 and 157), for both host and algal endosymbiont sequences. This makes the independent analysis of the data effectively impossible. It could be argued that the draft genomes used to separate the host and transcriptomic sequences are an important and critical piece of data in the experiment. Authors should either justify why the use of proprietary genomes is acceptable in this instance, or make the draft genomes available.

Do you have any ethical concerns with this paper?
No

Have you any concerns about statistical analyses in this paper?
No

Recommendation?
Major revision is needed (please make suggestions in comments)

Comments to the Author(s)
Major Comments:

Analyses make use of considerable unpublished genomic resources (see lines 147 and 157), for both host and algal endosymbiont sequences. This makes the independent analysis of the data effectively impossible. The draft genomes used to separate the host and transcriptomic sequences are an important and critical piece of data in the experiment. Authors should either justify why the use of proprietary genomes is acceptable in this instance, or make the draft genomes available.

The paper contains a massive amount of data and I fully understand the challenge of synthesizing the results. While many patterns are reported on, the manuscript would be dramatically improved if efforts were made to synthesize these results into larger models of the processes at hand. The results could perhaps be better communicated if differentially expressed immune components, for instance, were shown mapped (graphically, in figures) to their respective pathways.

Further, the manuscript navigation could benefit from subheadings in the discussion.

Minor Omments:

Line 49 - comma after "cells"

Line 51 - used "normal" twice; reword to avoid redundancy

Lines 60 - 62 - citations needed.

Line 71 - comma is unnecessary

Line 74 - citation needed

Line 88 - would be appropriate to include these data, either as a supplement or as a figure

Line 104 - inclusion of images (ideally with calibration reference) would be valuable

Line 111 - I'm slightly concerned regarding the time between fragment removal and snap freezing.

Line 136 - Remove "eliminates, or".

Line 138 and Line 171 - BLAST is a search algorithm. Against which database(s) did you use BLAST to search? Please further elaborate regarding the GO annotation performed. Which components of PANTHER DB were used? In Line 171 Blast2GO is used. Please unify and consider combining the annotation / gene ontology portions of the methods.

Line 171 - I believe that the test performed within Blast2GO for enrichment is a Fisher's exact test - this should be stated.

Line 176-183 - The paragraph is awkwardly worded and would benefit from considerable reworking.

Line 181 - Change "Denature" to "Denaturing."

Line 210 - Insert period after "transcriptome."

Line 260 - Are the mentioned GO terms actually statistically overrepresented? This can be examined via a hypergeometric test of differentially expressed GO terms against the background (all GO terms from all contigs).

Line 349 - Too colloquial, consider rewording.

Decision letter (RSOS-140214)

07-Oct-2014

Dear Dr Pinzon,

The Subject Editor assigned to your paper ("Whole transcriptome analysis reveals changes in expression of immune related genes during and after bleaching in a reef-building coral") has now received comments from reviewers. We would like you to revise your paper in accordance with the referee and Subject Editor suggestions which can be found below (not including confidential reports to the Editor). Please note this decision does not guarantee eventual acceptance.

Please submit a copy of your revised paper within 14 days - if we do not hear from you within this time then it will be assumed that the paper has been withdrawn. In exceptional circumstances, extensions may be possible if agreed with the Editorial Office in advance. Once

submitted your paper may be returned to the previous referees, or new ones if these are unavailable.

To revise your manuscript, log into http://mc.manuscriptcentral.com/bl and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions," click on "Create a Revision." Your manuscript number has been appended to denote a revision. Revise your manuscript and upload a new version through your Author Centre.

When submitting your revised manuscript, you must respond to the comments made by the referees and upload a file "Response to Referees" in "Section 6 - File Upload". Please use this to document how you have responded to the comments, and the adjustments you have made. In order to expedite the processing of the revised manuscript, please be as specific as possible in your response.

Once again, thank you for submitting your manuscript to Royal Society Open Science and I look forward to receiving your revision. If you have any questions at all, please do not hesitate to get in touch.

Yours sincerely,
Charlotte Wray
Senior Publishing Editor, Royal Society Open Science
openscience@royalsociety.org

Author's Response to Decision Letter for (RSOS-140214)

See Appendix A.

Appendix A

UNIVERSITY OF
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Department of Biology

Dr. Kevin Padian
Biology Editor
Royal Society Open Science

November 1, 2014

Dear Dr. Padian,

Please accept the revision of our manuscript "Whole transcriptome analysis reveals changes in expression of immune related genes during and after bleaching in a reef-building coral," for consideration of publication at the Royal Society Open Science Journal. I would like to thank you and the reviewers for the invaluable comments and suggestions that have improved the manuscript. Please find attached the revised manuscript and the responses to the reviewer's and editor comments.

We have addressed all of the comments and suggestions of the reviewers in this revision of the manuscript. A major concern was the unpublished data we were presenting in this paper. We have included some of the data (temperature profile of the region and *Symbiodinium* spp. genomic data) and pointed the reader to the source of other resources (*O. faveolata* genome). Other changes include a paragraph in the Methods section explaining our annotation and gene ontology procedure, figures with colony pictures and the cited pathways, clarifications in some of the ideas and minor suggestions of appropriate language. We have also added/corrected some sentences to improve the flow of the manuscript.

Below you will find a table with the detailed responses to all the comments. Each row includes the original comment and our response. When appropriate, the line numbers in the manuscript are included.

We appreciate your time and consideration.

Sincerely,

Jorge H. Pinzón C.

Department of Biology, The University of Texas at Arlington, 501 S. Nedderman Drive, Life Science Building, RM337, Arlington, TX 76019-0498, T (817)272-2872, Fax (817)272-2855, http://www.uta.edu/biology

Editor	
The authors should respond to the reviewers comments in particular reviewer 2 who brings up the issue of using unpublished data which are important for the manuscript.	Thanks for your comment. We realize this concern is important too and have added the data to the manuscript (i.e. Temperatures as a figure and the *Symbiodinium* genomic data in the form of an ESM file), or point the reader the appropriate source (i.e. *O. faveolata* consortium genome website)
Reviewer 1	
• Abstract and introduction are well written. They provide the necessary background to put the presented experiments into context. Their experimental subjects are appropriate to test their hypothesis since these are naturally bleached O. faveolata colonies compared to non-bleached colonies during the same environment and same time period. The value of this project lies in the fact that the results that they got are very close to what happens in nature so this information can be directly used to design conservation strategies and management plans.	Thanks for your comments we really appreciate them.
• One question I had right from the start was related to the authors' comments on corals of the same species but living in warmer vs. colder environments and how the warmer colonies are more resilient than the colder ones. Is this something that they plan to test in the future since they spend a paragraph on this topic? This is not addressed again in the rest of the manuscript. I would also be interested to if in the long-term, these previously bleached but now recovered corals are more susceptible to disease or bleaching; does a bleaching renders them immunologically suppressed for as long as the colony is alive? Would like to see this point discussed in the discussion.	Indeed these are very interesting questions that are worth pursuing in the future. In fact we have such experiments conducted and are currently analyzing them, but the data are beyond the scope of this project and manuscript.
• Typo: Page 6, line 128 should say "manufacturer's" instead of manufacture's	Thanks, it has been fixed.
• Methodology is very clear and also well	Thank you for your comment.

written. RNA-Seq is highly technical but authors did a good on presenting essential information in an easy to understand manner.	
• Page 9, lines 180-183: What is the purpose of the additional samples collected in Dec. 2010 and August 2011? I didn't find them used in the rest of the experiment but maybe I missed it.	These samples were collected to monitor changes in *Symbiodinium* species through the bleaching event. We used that information (at least from August 2011) in the discussion. Line 246-254 Section: "Effects of coral bleaching and the response of the coral holobiont"
• Page 11, paragraph starting in line 235: To clarify, do authors mean that during the sampling period the transcriptome of Symbiodinium of bleached and unbleached did not change the whole time yet the species associated while the bleached corals transcriptome did change? Or that the changes were the same as those seen in the metatranscriptome and that the metatranscriptome did change as discussed in previous paragraph?	Analyzes of the "Symbiodinium" and "other-eukaryotes" transcriptomes revealed that both have similar patterns to those seen in the "metatranscriptome," and appear to be associated with the identity of the symbionts present in the colony. This sentence (Bleached colonies showed changes in the dominant symbiotic species contrary to non-bleached colonies were the association was stable during the sampling period. – Lines 246-254) was added to make this point more clear.
• Page 13 line 267: annotated genes seem to return to normal expression levels a year after the bleaching event (no difference between bleach vs. unbleached)? Or is it that what was differentially expressed in Nov. 2010, returned to normal in March 2011, but were again differentially expressed almost a year later? This paragraph is a bit confusing. Also, if I am understanding the data correctly, I'm wondering if the return to differentially expressed genes a year later predates a future bleaching event or the onset of disease after October 2011. I'm sure the authors are continuing to monitor these colonies and hopefully they'll follow up on their status. Is it possible to comment on the health of those colonies (at least visually) for this manuscript? If not, this wouldn't disqualify this manuscript for publication; I'm just curious.	The expression levels for these genes in October 2011, were similar to those during the bleaching event, even tough some of them were back to "normal" (by comparison with non-bleached colonies) in March 2011. We did not collect these colonies before the bleaching, which means that we do not have a "baseline" expression level from before. Colonies in October 2011 appear "healthy" with no signs of bleaching. A clarification has been added to the end of this sentence (Line 283).
• Page 15 paragraph on line 315. When authors refer to the holobiont expression profile I am assuming that it is the same as	This particular paragraph is dealing with expression levels of single genes. The reviewer however pointed out a possible misunderstanding

the metatranscriptome? But expression of bleached colonies grouped Nov 10 and Oct 11 together although not March 11, yet everything else part of the holobiont (Symbiodinium and other eukaryotes) grouped with the unbleached metatranscriptome or the metranscriptome as a whole (bleached + unbleached)? Please clarify this paragraph a little better.	that was fixed by changing this part of the paragraph to: The expression of TRAF1, MATL1, and ETV3, is interesting in that these genes do not follow the patterns seen in the other immune-related genes. The TRAF1 deviated from the expression levels seen in the other tumor necrosis factor pathway genes, as it is up-regulated in the bleached colonies. MATL1, was also up-regulated in bleached colonies, and has high expression levels in October 2011. Finally, ETV3 through the survey appeared as a low expressed gene in bleached colonies, compared to unbleached colonies, but in the last month (October 2011) its expression increased to higher levels than those seen in the unbleached colonies (Figure 8). Lines 310-316.
• The Mydlarz lab specializes in work at the protein level so I am wondering if the authors plan to look at the expression of some of these genes at the protein level to see if they correlate. There's a lot of regulation that happens post-translationally and I am wondering if they plan to look at this.	This is part of the plan, but due to the large amount of data, will have to be reserved for another manuscript.
• Overall this was an excellent paper and I enjoyed reading it very much.	Again, thanks for your kind comments; we are glad you enjoy our manuscript.
Reviewer 2	
Analyses make use of considerable unpublished genomic resources (see lines 147 and 157), for both host and algal endosymbiont sequences. This makes the independent analysis of the data effectively impossible. The draft genomes used to separate the host and transcriptomic sequences are an important and critical piece of data in the experiment. Authors should either justify why the use of proprietary genomes is acceptable in this instance, or make the draft genomes available.	The *Symbiodinium* genomic data from Dr. LaJeunesse is going to be supplied as supplementary material in a file combining all the *Symbiodinium* resources used in the manuscript. This is now in part of the manuscript. The coral genome is currently available at http://montastraea.psu.edu. We have made the appropriate changes in the manuscript to reflect the availability of this information.
The paper contains a massive amount of data and I fully understand the challenge of synthesizing the results. While many patterns are reported on, the manuscript	We understand this point and we acknowledge that we do present a massive amount of data. It is a difficult balance to present all the interesting patterns in a synthetic and manageable unit. We

would be dramatically improved if efforts were made to synthesize these results into larger models of the processes at hand.	appreciate your ideas to map the immune components to their respective pathways and have added this (see below) to address this comment as well.
The results could perhaps be better communicated if differentially expressed immune components, for instance, were shown mapped (graphically, in figures) to their respective pathways.	This is a great idea. Figure 7 with pathways including most of the immune-related genes has been added.
Further, the manuscript navigation could benefit from subheadings in the discussion.	Thanks for your comments. Indeed the addition of four subheadings to the discussion improved the flow of the manuscript.
Line 49 - comma after "cells"	Added
Line 51 - used "normal" twice; reword to avoid redundancy	Second “normal” change with “typical”
Lines 60 - 62 - citations needed.	Two references added: Croquer A., Weil E. 2009 Changes in Caribbean coral disease prevalence after the 2005 bleaching event. *Dis Aquat Org* **87**(1-2), 33-43. Brandt M.E., McManus J.W. 2009 Disease incidence is related to bleaching extent in reef-building corals. *Ecology* **90**(10), 2859-2867. (doi:10.1890/08-0445.1).
Line 71 - comma is unnecessary	Removed
Line 74 - citation needed	Two references added: DeSalvo M.K., Sunagawa S., Voolstra C.R., Medina M. 2010 Transcriptomic responses to heat stress and bleaching in the elkhorn coral *Acropora palmata*. *Mar Ecol Prog Ser* **402**, 97-113. (doi:10.3354/meps08372). Desalvo M.K., Voolstra C.R., Sunagawa S., Schwarz J., Stillman J., Coffroth M.A., Szmant-Froelich A.M., Medina M. 2008 Differential gene expression during thermal stress and bleaching in the Caribbean coral *Montastraea faveolata*. *Mol Ecol* **17**(17), 3952-3971.
Line 88 - would be appropriate to include these data, either as a supplement or as a figure	Figure 1 depicting the thermal anomaly related to the 2010-bleaching event has been added.
Line 104 - inclusion of images (ideally with calibration reference) would be valuable	A figure with bleached and non-bleached colonies has been added (Figure 2).
Line 111 - I'm slightly concerned regarding the time between fragment removal and snap freezing.	All samples were maintained and transported in seawater at the temperature of the collection site. The time between collection and freezing was kept

	to the absolute minimum needed to accomplish these goals and was the same throughout all the collections. This information has been added to the text.
Line 136 - Remove "eliminates, or".	Removed
Line 138 and Line 171 - BLAST is a search algorithm. Against which database(s) did you use BLAST to search? Please further elaborate regarding the GO annotation performed. Which components of PANTHER DB were used? In Line 171 Blast2GO is used. Please unify and consider combining the annotation / gene ontology portions of the methods.	Thanks for your comment. These concerns were addressed this paragraph added to the end of section 2.4. Gene expression analysis and gene ontology (Lines 176-186): Gene ontology annotations were initially determined using blast [50] for the metatranscriptome contigs/genes, and further explored with Protein Analysis Through Evolutionary Relationships (PANTHER) [51] and Blast2GO [52] for genes showing significant gene expression differences (corrected p-values > 0.05). The metatranscriptome was blasted against the Swiss-Prot database. In Blast2Go the annotations were obtained from the NCBI's nucleotide database, InterPro, GO, Enzyme Codes and KEGG. Enrichment test among the differentially expressed genes were performed for the biological processes using the Fisher's Exact Test on Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.7 [53]. All biological processes, except locomotion and biological regulation show p-values smaller than 0.05), suggesting that the processes are significantly enriched. Pathways involving genes with significant differences were obtained using PathVisio [54] from WikiPathways [55] and the Pathway Interaction Database [56].
Line 171 - I believe that the test performed within Blast2GO for enrichment is a Fisher's exact test - this should be stated.	We performed the enrichment test on DAVID. This was added to the gene ontology section of Methods
Line 176-183 - The paragraph is awkwardly worded and would benefit from considerable reworking.	Modified to (lines 188-194): The identity of the associated *Symbiodinium* types in each sample was determined with BLAST [50]. Reads from each of the samples were aligned against sequences of the internal transcribed spacer 2 (ITS2) of *Symbiodinium* types known to inhabit *O. faveolata* (*S. fitti*, D1a, B1, C3, C3d, C3e, C7, C12). Alignments with 100% match were

	use as the correct identity. The symbiont identity of additional samples of the same colonies but collected in other months (September and December 2010 and August 2011), was determined using Denaturing Gradient Gel Electrophoresis of the ITS2 region [57-62]
Line 181 - Change "Denature" to "Denaturing."	Changed.
Line 210 - Insert period after "transcriptome."	Inserted
Line 260 - Are the mentioned GO terms actually statistically overrepresented? This can be examined via a hypergeometric test of differentially expressed GO terms against the background (all GO terms from all contigs).	We performed the enrichment test on DAVID. The overrepresentation of some of the processes has been noted in the manuscript (Lines 182-184).
Line 349 - Too colloquial, consider rewording	Sentence modified to (Lines 360-362): However, a group of genes involved in protein synthesis and transport were up-regulated in the bleached colonies one year after bleaching, perhaps in an effort to overcompensate the observed down-regulation observed during the bleaching event.