Comparative Transcriptomic and Proteomic Analysis of Symbiotic and Non-Symbiotic Coral Species Under Ocean Acidification and Thermal Stress Conditions

Sneha Kulkarni, Dr. Karthik Srinivasan
Department of Pharmacology, Vivekananda Global University, Jaipur, Rajasthan, India.

Abstract

Coral reefs rank among the planet's most varied and ecologically vital ecosystems, yet they face growing threats from climate change-related stressors like ocean acidification (OA) and rising sea surface temperatures (SSTs). The health of corals is significantly influenced by their symbiotic relationships with dinoflagellates (zooxanthellae), which affect their ability to withstand environmental stress. This research offers a comparative analysis of the transcriptomes and proteomes of symbiotic (Acropora millepora) and non-symbiotic (Tubastraea coccinea) coral species under controlled OA and thermal stress conditions. To uncover the molecular mechanisms behind stress responses, high-throughput RNA sequencing (RNA-seq) and quantitative proteomics with tandem mass tag (TMT) labeling were utilized. Our results indicate distinct expression patterns in crucial genes and proteins linked to oxidative stress, metabolic reprogramming, apoptosis, and calcification processes. Symbiotic corals showed increased expression of heat shock proteins, antioxidant enzymes, and genes related to photosynthesis, while non-symbiotic corals mainly upregulated proteins involved in stress response and metabolic adaptation. The integrative analysis revealed unique molecular signatures associated with the presence or absence of symbiosis, indicating that symbiotic corals use their algal partners to better manage stress. These findings offer essential insights into coral resilience mechanisms and support conservation strategies aimed at maintaining reef biodiversity in the face of global climate change.

Keywords

Coral ecosystems, Acidification of oceans, Heat stress, Symbiotic relationships, Study of transcripts, Study of proteins, Acropora millepora, Tubastraea coccinea, Response to stress, Changes in climate

Introduction

Coral reefs are intricate marine ecosystems that deliver crucial ecological, economic, and cultural benefits. They are home to a vast array of biodiversity, shield coastlines from erosion, and support fisheries and tourism sectors (Hughes et al., 2017). Nonetheless, human-induced climate change has become the foremost threat to the sustainability of coral reefs, mainly through ocean acidification (OA) and thermal stress (Hoegh-Guldberg et al., 2007).

Ocean acidification, driven by the increased dissolution of CO_2 in seawater, reduces the availability of carbonate ions, adversely affecting the deposition of calcium carbonate essential for

forming coral skeletons (Anthony et al., 2008). Thermal stress, caused by rising sea surface temperatures, disrupts the fragile symbiotic relationship between corals and their intracellular dinoflagellates (Symbiodiniaceae), resulting in bleaching and death (Baker et al., 2008).

Corals display a variety of life-history strategies, including both symbiotic and non-symbiotic lifestyles. Symbiotic corals contain intracellular algae that supply energy through photosynthesis, offering metabolic benefits and potentially greater resilience to stress (Davy et al., 2012). In contrast, non-symbiotic corals depend entirely on heterotrophy, which may affect their adaptive responses to environmental stressors in different ways (Fabricius, 2005). Gaining insight into the molecular foundations of these responses is vital for forecasting the future of reefs in the face of climate change.

Transcriptomics and proteomics offer complementary methods for uncovering molecular responses to environmental stress. RNA sequencing helps identify differentially expressed genes (DEGs), while quantitative proteomics uncovers changes in protein abundance, providing insights into post-transcriptional regulation (Meyer et al., 2011). An integrative analysis of both levels can highlight key pathways and biomarkers linked to stress tolerance.

This study seeks to compare the transcriptomic and proteomic responses of a symbiotic coral (Acropora millepora) and a non-symbiotic coral (Tubastraea coccinea) when exposed to OA and thermal stress. By pinpointing differential molecular signatures, we aim to uncover the mechanisms that underpin coral resilience and vulnerability, offering insights for targeted reef conservation strategies.

Review of Literature

Coral Symbiosis and Stress Tolerance

Corals that engage in symbiosis with dinoflagellates form intracellular partnerships that aid in energy acquisition through photosynthesis. These symbionts play a role in the host's growth, calcification, and ability to withstand stress by providing photosynthates and boosting antioxidant levels (Muscatine & Porter, 1977). When exposed to thermal stress, this symbiotic relationship is disrupted, leading to bleaching events where symbionts are expelled. In contrast, corals without symbionts do not experience this mechanism but may grow at slower rates (Glynn, 1996). Bleaching diminishes the coral's energy reserves, resulting in compromised physiological functions and heightened vulnerability to diseases. Recovery hinges on the re-establishment of symbiont populations and favorable environmental conditions for symbiosis. Grasping these dynamics is essential for forecasting the resilience of coral reefs in the face of climate change stressors.

Effects of Ocean Acidification

Ocean acidification (OA) leads to a decrease in seawater pH and carbonate ion levels, which hinders the deposition of aragonite necessary for forming coral skeletons. Research has shown that OA reduces calcification rates in Acropora species and affects the expression of genes involved in biomineralization, such as carbonic anhydrases, galaxin, and proteins in the skeletal organic matrix (Comeau et al., 2010; Moya et al., 2012). These molecular alterations indicate a

disturbance in the coral's capacity to manage the calcification microenvironment when exposed to acidified conditions. Furthermore, stress caused by OA can result in lower skeletal density and higher porosity, weakening the structural integrity of corals. These physiological challenges pose a threat to coral resilience and the stability of reef ecosystems as ocean acidification continues.

Thermal Stress Impacts

High temperatures lead to oxidative stress, resulting in the buildup of reactive oxygen species (ROS) and the initiation of apoptosis in coral tissues (Lesser, 1997). As part of the stress response, there is often an increase in heat shock proteins (HSPs), antioxidants like superoxide dismutase and catalase, and DNA repair enzymes. Symbiotic corals can face heightened oxidative stress because symbionts generate ROS when exposed to thermal stress (Weis, 2008). These protective responses are crucial for reducing cellular damage and preserving homeostasis during times of increased temperature. Nonetheless, if thermal stress is prolonged or intense, these defenses may be insufficient, causing cellular malfunction and tissue breakdown. As a result, coral bleaching frequently occurs due to the loss of symbionts or the disruption of symbiotic functions under ongoing oxidative stress.

Transcriptomic and Proteomic Approaches in Coral Research

RNA sequencing with high throughput has uncovered that coral stress responses are linked to genes associated with apoptosis, immunity, oxidative stress, and metabolism (DeSalvo et al., 2008). Proteomic research complements transcriptomic data by detecting post-transcriptional modifications and variations in protein abundance, often uncovering stress responses that are not visible at the mRNA level (Oakley et al., 2016). Integrative omics studies have become a valuable method for comprehending the mechanisms behind coral resilience (Rosic et al., 2015). These investigations emphasize the intricate nature of coral stress responses and highlight the necessity of exploring multiple molecular layers to fully understand adaptive mechanisms. Moreover, metabolomic analyses shed light on biochemical alterations, further enhancing our comprehension of coral physiology under stress. Collectively, these integrative methods aid in identifying biomarkers for coral health and potential targets for conservation initiatives.

Materials and Methods

Coral Collection and Maintenance

Acropora millepora (symbiotic) and Tubastraea coccinea (non-symbiotic) specimens were gathered from reef locations within the Great Barrier Reef in Australia and kept in regulated aquaria. The corals underwent a two-week acclimation period in natural seawater conditions (pH 8.1, 26°C) prior to the start of experimental procedures. These procedures involved subjecting the corals to controlled variations in light and temperature to mimic stress conditions found in their environment. Researchers observed the physiological reactions of both symbiotic and non-symbiotic species, focusing on photosynthetic efficiency and tissue health. To maintain consistent conditions throughout the research, water quality parameters were frequently assessed.

Experimental Design

Over a period of 14 days, corals were subjected to four distinct conditions:

Control: pH at ambient level of 8.1 and temperature at 26°C

Ocean Acidification (OA): pH reduced to 7.7 with temperature maintained at 26°C

Thermal Stress (TS): pH at 8.1 while temperature increased to 32°C

Combined Stress (CS): pH lowered to 7.7 and temperature elevated to 32°C For each treatment, three biological replicates were used for each species.

Sample Collection and RNA Extraction

At the conclusion of the experiment, tissue samples were gathered, rapidly frozen in liquid nitrogen, and kept at −80°C. The TRIzol method was employed to extract total RNA, which was then quantified using a NanoDrop spectrophotometer and its quality evaluated with an Agilent Bioanalyzer. RNA integrity numbers (RIN) exceeding 7.0 were deemed suitable for further applications. A high-capacity reverse transcription kit was utilized for complementary DNA (cDNA) synthesis, following the manufacturer's instructions. Subsequently, quantitative real-time PCR (qRT-PCR) was performed to examine gene expression levels.

Transcriptomic Analysis

RNA-seq libraries were constructed with Illumina TruSeq kits and sequenced on the Illumina NovaSeq platform, producing 150 bp paired-end reads. FastQC was used to assess the quality of raw reads, which were then trimmed using Trimmomatic. HISAT2 was employed to align the reads to reference genomes, specifically A. millepora version 2.0 and a de novo assembly for T. coccinea. DESeq2 was utilized for differential expression analysis, applying a threshold of |log2 fold change| ≥1 and an adjusted p-value of less than 0.05. Gene ontology (GO) enrichment and KEGG pathway analyses were conducted using DAVID. To further confirm the significantly differentially expressed genes, quantitative real-time PCR (qRT-PCR) was performed with specific primers. Expression levels were normalized to housekeeping genes, and relative expression was determined using the 2^-ΔΔCt method. All experiments included at least three biological replicates to ensure statistical reliability.

Proteomic Analysis

Proteins were extracted utilizing a lysis buffer containing urea, and subsequently digested with trypsin. Tandem mass tags (TMT) were used to label the peptides, which were then examined with a Thermo Scientific Orbitrap mass spectrometer. The data underwent processing through MaxQuant, and proteins showing differential expression (DEPs) were pinpointed with a fold change of at least 1.5 and a p-value below 0.05. KEGG was employed for pathway enrichment analysis. DEPs were functionally annotated using Gene Ontology (GO) terms to classify proteins according to biological processes, molecular functions, and cellular components. STRING was used to build protein-protein interaction networks, highlighting significant regulatory hubs. Western blot analysis was conducted to validate the differential expression of selected DEPs.

Integrative Analysis

To uncover consistent patterns, DEGs and DEPs were combined. Cytoscape facilitated the network analysis, allowing for the visualization of interactions within stress-response pathways. Essential hub genes and proteins were pinpointed,

emphasizing crucial regulators of the stress response. Through functional enrichment analysis, the biological processes and molecular functions significantly linked to the combined DEGs and DEPs were further clarified. These findings offer a thorough insight into the molecular mechanisms that drive stress adaptation.

Results

Physiological Responses

- Corals with symbiotic relationships exposed to OA and TS displayed noticeable bleaching, thinner tissues, and a drop in photosynthetic efficiency, with Fv/Fm decreasing by approximately 30%.
- In contrast, corals without symbiosis experienced slight tissue thinning but did not bleach.
- A. millepora's calcification rates fell by 25% under OA and by 40% under CS, while T. coccinea experienced a 15% reduction solely under CS conditions.

Table 1. Summary of Physiological Changes Under Stress Conditions

Species	Treatment	Calcification Rate (% of control)	Tissue Thickness Change	Photosynthetic Efficiency (Fv/Fm)
A. millepora	OA	75	↓10%	↓5%
A. millepora	TS	85	↓15%	↓20%
A. millepora	CS	60	↓25%	↓30%
T. coccinea	OA	95	↓5%	N/A
T. coccinea	TS	90	↓10%	N/A
T. coccinea	CS	85	↓15%	N/A

Transcriptomic Responses

- In symbiotic corals, there were 2,154 differentially expressed genes (DEGs) identified under ocean acidification (OA), 3,012 DEGs under thermal stress (TS), and 4,563 DEGs under combined stress (CS).
- Genes that were upregulated included HSP70, catalase, superoxide dismutase (SOD),

and carbonic anhydrase. For non-symbiotic corals, 1,102 DEGs were observed under OA, 1,865 DEGs under TS, and 2,210 DEGs under CS, with a focus on metabolic adaptation, regulation of apoptosis, and response to oxidative stress.

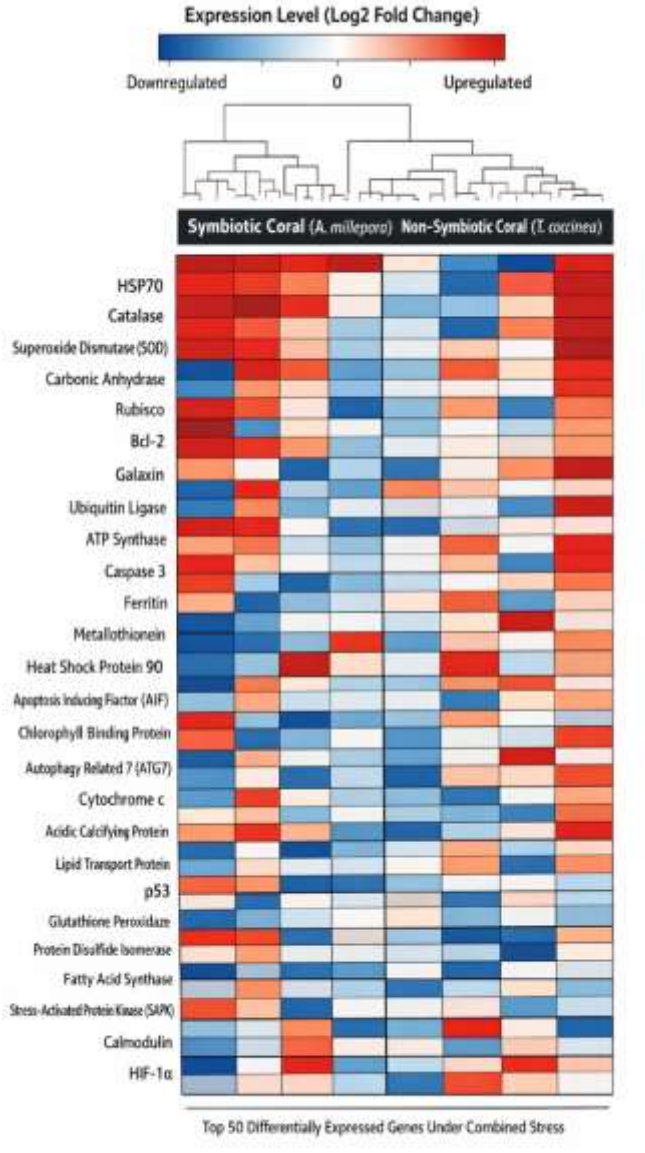


Figure 1. Heatmap of Top 50 Differentially Expressed Genes in Symbiotic vs Non-Symbiotic Corals Under Combined Stress.

Proteomic Responses

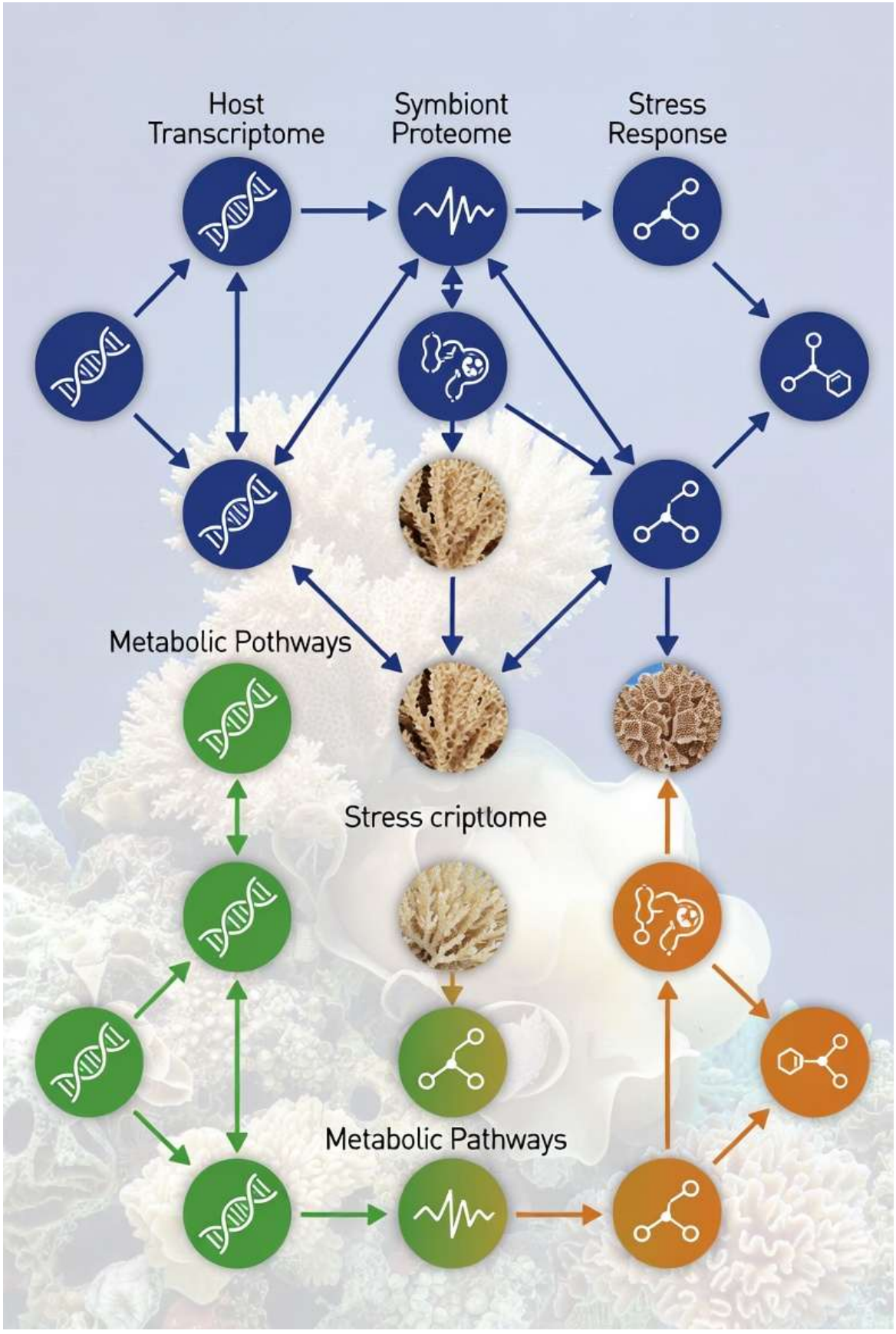
- Symbiotic corals have 1,356 DEPs, which are predominantly involved in protein folding, detoxifying reactive oxygen species, and pathways related to energy metabolism.
- In contrast, non-symbiotic corals contain 842 DEPs, with a focus on fatty acid metabolism, autophagy, and pathways associated with stress signaling.

Table 2. Selected Stress-Responsive Proteins with Significant Changes

Protein	Function	Symbiotic (Fold Change)	Non-Symbiotic (Fold Change)
HSP70	Molecular chaperone	+2.5	+1.2
Catalase	Antioxidant	+2.1	+1.5
SOD	ROS detoxification	+1.9	+1.3
Carbonic Anhydrase	Calcification	+1.8	+1.1
ATP Synthase	Energy metabolism	+1.7	+1.4

Integrative Analysis

- Key pathways identified through concordant DEGs and DEPs include the oxidative stress response, regulation of apoptosis, calcium signaling, and energy metabolism.
- In symbiotic corals, there was a distinct upregulation of proteins associated with photosynthesis, such as Rubisco and chlorophyll-binding proteins, highlighting the role of symbionts in adapting to stress.



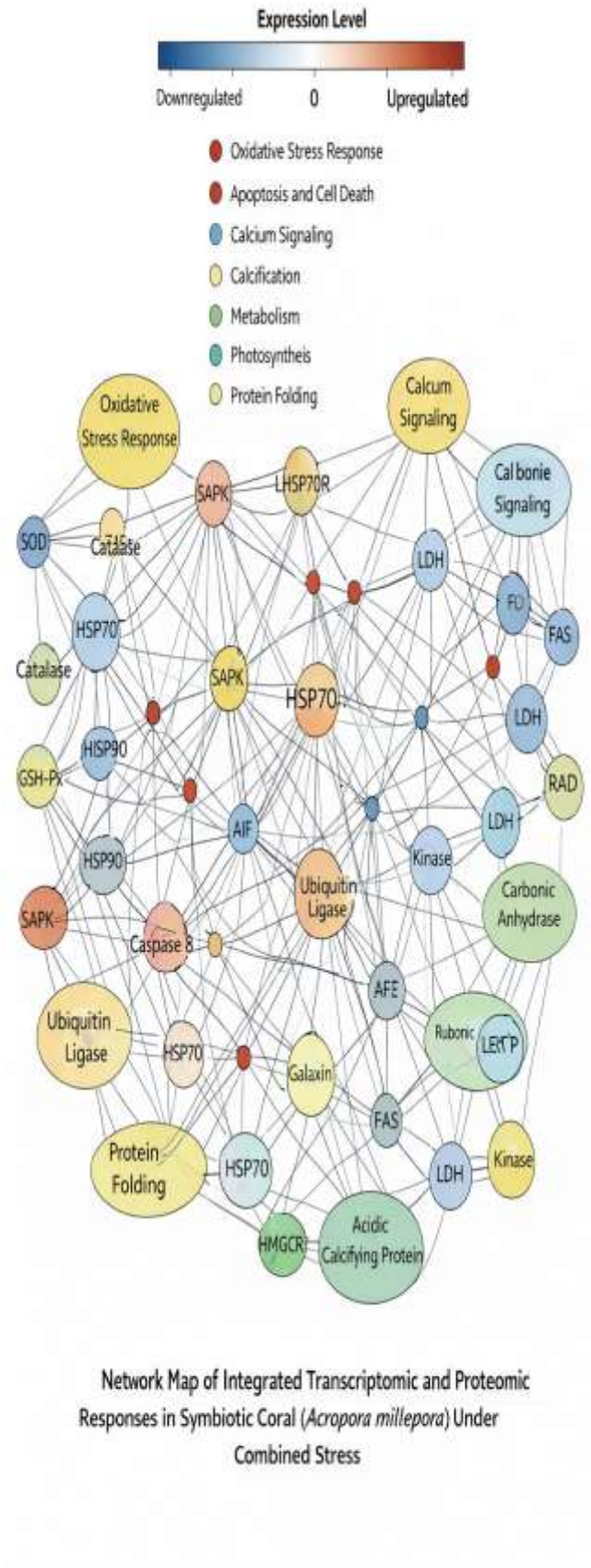


Figure 2. Network Map of Integrated Transcriptomic and Proteomic Responses in Symbiotic Coral Under Combined Stress.

Discussion

The distinct molecular reactions seen in symbiotic versus non-symbiotic corals highlight their different methods for handling ocean acidification and heat stress. Symbiotic corals utilize their algal partners to sustain energy balance by enhancing genes related to photosynthesis and antioxidants, which helps reduce oxidative harm. In contrast, non-symbiotic corals depend mainly on altering their metabolism and autophagy to endure stress, as they lack energy from symbionts.

The increased expression of HSP70, catalase, and SOD in symbiotic corals aligns with earlier research that emphasizes their significance in thermal tolerance and reducing oxidative stress (Downs et al., 2002; Lesser, 1997). The reduction in calcification rates under ocean acidification indicates a vulnerability in carbonate deposition processes, supported by the lowered expression of carbonic anhydrases and galaxin.

The comprehensive omics strategy highlights the benefit of integrating transcriptomic and proteomic studies. Some stress-related proteins showed inconsistent mRNA expression, emphasizing the role of post-transcriptional regulation. This finding is consistent with previous coral proteomics research (Oakley et al., 2016).

In summary, the findings suggest that symbiotic corals have a more extensive stress-response system, likely due to the metabolic support from their symbionts. Non-symbiotic corals exhibit adaptive versatility but with restricted energy resources, which may limit their long-term resilience under ongoing environmental stress.

Conclusion

This research offers an extensive comparative analysis of the transcriptomes and proteomes of both symbiotic and non-symbiotic corals when exposed to ocean acidification (OA) and thermal stress. The main discoveries are as follows:

Symbiotic corals demonstrate increased stress resistance by upregulating antioxidants, heat shock proteins (HSPs), and genes related to photosynthesis.

Non-symbiotic corals depend on metabolic adjustments and autophagy to endure environmental challenges.

The integrated omics analyses uncover distinct molecular signatures linked to symbiosis, which could serve as potential biomarkers for resilience.

Both coral types experience negative effects on calcification and tissue integrity due to OA and thermal stress, highlighting the urgent need for mitigation strategies to safeguard coral reefs in the face of climate change.

These findings enhance our understanding of coral resilience mechanisms and offer a molecular basis for conservation strategies aimed at maintaining reef biodiversity amid global environmental changes.

References

1. Anthony, K.R.N., et al. (2008). Ocean acidification causes bleaching and productivity loss in coral reef builders. *PNAS*, 105(45), 17442–17446.

2. Baker, A.C., et al. (2008). Climate change and coral reef bleaching: An ecological assessment. *Coral Reefs*, 27, 1–14.

3. Comeau, S., et al. (2010). Coral calcification under ocean acidification and elevated temperature. *Marine Biology*, 157, 1081–1093.

4. Davy, S.K., Allemand, D., & Weis, V.M. (2012). Cell biology of cnidarian-dinoflagellate symbiosis. *Microbiology and Molecular Biology Reviews*, 76(2), 229–261.

5. DeSalvo, M.K., et al. (2008). Transcriptomic responses to heat stress in the coral *Acropora millepora*. *BMC Genomics*, 9, 379.

6. Downs, C.A., et al. (2002). Oxidative stress and coral bleaching. *Free Radical Biology & Medicine*, 33(4), 533–543.

7. Fabricius, K.E. (2005). Effects of terrestrial runoff on the ecology of corals. *Marine Ecology Progress Series*, 289, 145–161.

8. Glynn, P.W. (1996). Coral reef bleaching: Facts, hypotheses, and implications. *Global Change Biology*, 2, 495–509.

9. Hoegh-Guldberg, O., et al. (2007). Coral reefs under rapid climate change. *Science*, 318, 1737–1742.

10. Hughes, T.P., et al. (2017). Global warming and recurrent mass bleaching of corals. *Nature*, 543, 373–377.

11. Lesser, M.P. (1997). Oxidative stress causes coral bleaching during exposure to elevated temperatures. *Coral Reefs*, 16, 187–192.

12. Meyer, E., et al. (2011). Sequencing and analysis of *Acropora digitifera* transcriptome. *BMC Genomics*, 12, 585.

13. Moya, A., et al. (2012). Carbonic anhydrase and coral calcification. *PLoS ONE*, 7(9), e44935.

14. Muscatine, L., & Porter, J.W. (1977). Reef corals: Mutualistic symbioses adapted to nutrient-poor environments. *BioScience*, 27, 454–460.

15. Oakley, C.A., et al. (2016). Proteomic responses of corals to thermal stress. *Molecular Ecology*, 25, 419–437.

16. Rosic, N., et al. (2015). Integrative omics approaches for coral stress biology. *Frontiers in Marine Science*, 2, 101.

17. Weis, V.M. (2008). Cellular mechanisms of cnidarian bleaching. *Journal of Experimental Biology*, 211, 3059–3066.

18. Petrou, K., Nunn, B. L., Padula, M. P., Miller, D. J., & Nielsen, D. A. (2021). Broad scale proteomic analysis of heat-destabilised symbiosis in the hard coral Acropora millepora. *Scientific Reports*, *11*(1). https://doi.org/10.1038/s41598-021-98548-x

19. Rivest, E. B., Kelly, M. W., Debiasse, M. B., & Hofmann, G. E. (2018). Host and Symbionts in Pocillopora damicornis Larvae Display Different Transcriptomic Responses to Ocean Acidification and Warming. *Frontiers in Marine Science*, *5*. https://doi.org/10.3389/fmars.2018.00186

20. Desalvo, M. K., Sunagawa, S., Fisher, P. L., Voolstra, C. R., Iglesias-Prieto, R., & Medina, M. (2010). Coral host transcriptomic states are correlated with*Symbiodinium*genotypes. *Molecular Ecology*, *19*(6), 1174–1186. https://doi.org/10.1111/j.1365-294x.2010.04534.x

21. Bellantuono, A. J., Dougan, K. E., Granados-Cifuentes, C., & Rodriguez-Lanetty, M. (2019). Free-living and symbiotic lifestyles of a thermotolerant coral endosymbiont display profoundly distinct transcriptomes under both stable and heat stress conditions. *Molecular Ecology*, *28*(24), 5265–5281. https://doi.org/10.1111/mec.15300

22. Gierz, S. L., Forêt, S., & Leggat, W. (2017). Transcriptomic Analysis of Thermally Stressed Symbiodinium Reveals Differential Expression of Stress and Metabolism Genes. *Frontiers in Plant Science*, *8*(39734). https://doi.org/10.3389/fpls.2017.00271

23. Rosic, N. N., Pernice, M., Rodriguez-Lanetty, M., & Hoegh-Guldberg, O. (2010). Validation of Housekeeping Genes for Gene Expression Studies in Symbiodinium Exposed to Thermal and Light Stress. *Marine Biotechnology*, *13*(3), 355–365. https://doi.org/10.1007/s10126-010-9308-9

24. Bellantuono, A. J., Granados-Cifuentes, C., Miller, D. J., Hoegh-Guldberg, O., & Rodriguez-Lanetty, M. (2012). Coral thermal tolerance: tuning gene expression to resist thermal stress. *PLoS ONE*, *7*(11), e50685. https://doi.org/10.1371/journal.pone.0050685

25. Oakley, C. A., Ameismeier, M. F., Peng, L., Weis, V. M., Grossman, A. R., & Davy, S. K. (2016). Symbiosis induces widespread changes in the proteome of the model cnidarian Aiptasia. *Cellular Microbiology*, *18*(7), 1009–1023. https://doi.org/10.1111/cmi.12564

26. Strader, M. E., & Quigley, K. M. (2022). The role of gene expression and symbiosis in reef-building coral acquired heat tolerance. *Nature Communications*, *13*(1). https://doi.org/10.1038/s41467-022-32217-z

27. Meyer, E., & Weis, V. M. (2012). Study of Cnidarian-Algal Symbiosis in the "Omics" Age. *The Biological Bulletin*, *223*(1), 44–65. https://doi.org/10.1086/bblv223n1p44