

氏 名 岸本 真理子

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学位論文題目 Study on the thermal sensitivity of cnidarian-algal symbiosis
using Aiptasia model system

論文審査委員 主 査 教授 上野 直人

准教授 高橋 俊一

教授 川口 正代司

准教授 新里 宙也

東京大学 大気海洋研究所 分子海洋生物学分野

(Form 3)

Summary of Doctoral Thesis

Name in full Kishimoto, Mariko

Title Study on the thermal sensitivity of cnidarian-algal symbiosis using *Aiptasia* model system (セイタカイソギンチャクモデル実験系を用いた刺胞動物と褐虫藻の共生関係とその温度感受性の研究)

Since 1980s, the coverage of coral reefs has been dramatically decreasing worldwide due to ongoing environmental changes. Previous studies have demonstrated that the coral-algal symbiosis is sensitive to moderate heat stress, resulting in the loss of symbiotic algae, i.e. bleaching, and subsequent mortality. However, the mechanism of heat stress-induced dissociation of symbiotic relationship remains unknown, although it has been intensively studied in the last few decades. In this study, using sea anemone *Aiptasia* as a model system, the effect of heat stress on the establishment of cnidarian-algal symbiosis, the maintenance of the symbiosis and the re-establishment of the symbiosis after bleaching was examined.

In the first chapter of this thesis, the effect of heat stress on the establishment of symbiosis was investigated. The process of algal uptake by cnidarians has several steps including the acquisition of free-living algae from environment, the retention of algal cells in gastrovascular cavity, the transportation of algal cells to endoderm tissue and the engulfment of algal cells to host cells. It has been reported that heat stress suppresses the efficiency of symbiosis, but the mechanism behind this suppression has remained unknown. This study demonstrated that heat stress suppresses the infectivity of algae to host cnidarians, resulting in the failure to establish symbiosis under increased temperatures. When algal cells from a normal temperature were introduced into *Aiptasia* polyps, almost all cells were retained in the polyps and gradually spread through the polyps within few days. On the other hand, when heat-treated algae were introduced, algal cells were expelled from the polyps before they spread through the

body. These results suggest heat stress on algae induces active expulsion by host *Aiptasia*, resulting in suppressing the establishment of symbiosis. The experiments were repeated with another algal strain. With the second algal strain, no significant effect of heat stress on the infectivity was observed. These results suggest that the algal infectivity to *Aiptasia* varies by algal type under heat stress.

In the second chapter of this thesis, the effect of heat stress on the maintenance of cnidarian-algal symbiosis was investigated. Algal density in cnidarians is maintained by the dynamic balance between algal uptake and release of them at their tissues. In the current hypothesis, heat stress accelerates the algal release from host cells, resulting in the acceleration of algal expulsion from host polyps and decrease of algal density. However, suppression of algal uptake into host cells can also accelerate the algal expulsion from host polyps and decrease the algal density. Yet this possibility has not been experimentally examined. In this study, I used two algal strains which show different thermal sensitivities: one possesses infectivity under heat stress and the other does not. When *Aiptasia* polyps had the heat-sensitive algae, the algal density declined by increase in the water temperature with more algal expulsion from host polyps. However, the algal density and number of algae expelled from the host polyps did not change by increase in temperature when *Aiptasia* had heat-tolerant algae. These results suggest that the thermal sensitivity of algal infectivity decides if they can maintain the symbiotic relationship under heat stress. Furthermore, these results suggest that the heat-tolerant algae bring higher bleaching tolerance under elevated temperatures.

In the third chapter of this thesis, the effect of heat stress on the potential of corals to recover from bleaching was investigated. Bleaching can cause mortality of corals; however, it is not always lethal because corals can recover the density of algae through re-uptake of algal cells. However, in the last few decades, the

coverage of corals has dramatically decreased in many regions, implying that such recovery from bleaching is often limited in the field. The limiting factors have remained unknown. In this study, it was shown that heat-treated algae lost infectivity and that it was reversible. However, long-term exposure to heat stress slowed the recovery of their infectivity. These results suggest that the loss of algal infectivity following exposure to heat stress can be a factor limiting the recovery from bleaching.

In this study, I discovered that (1) heat stress suppresses the infectivity of algae, (2) thermal sensitivity of the infectivity varies by algal strain, (3) suppression of algal infectivity can cause the loss of algal density (bleaching) and (4) recovery of cnidarians from bleaching can be limited by suppressed infectivity of algae. Overall, my study demonstrates that the loss of algal infectivity upon the heat stress can be a major problem for corals by suppressing the establishment of coral-algal symbiosis, inducing the dissociation of coral-algal symbiotic relationship (bleaching) and limiting recovery from bleaching. These findings bring new insights into how increased temperatures have damaged the coral-algal symbiotic relationships in last few decades in coral reefs and how corals can adapt to ongoing global warming.

博士論文審査結果

Name in Full
氏名 岸本 真理子

Title
論文題目 Study on the thermal sensitivity of cnidarian-algal symbiosis using Aiptasia model system

刺胞動物であるセイタカイソギンチャクは褐虫藻を細胞内に取り込むことで、褐虫藻の光合成産物を栄養として得る共生を営んでおり、褐虫藻との共生の破綻が主な原因と考えられる造礁サンゴの白化メカニズムの研究の良いモデルとなっている。サンゴの白化の原因は海水温の上昇と考えられていることから、出願者の岸本さんは、共生する褐虫藻および宿主となるセイタカイソギンチャクの温度感受性と共生関係の破綻メカニズムについて研究を行った。まず、宿主であるセイタカイソギンチャクの胃腔に褐虫藻を加え、共生に必要な内胚葉細胞が存在する触手中に取り込まれた褐虫藻の数をクロロフィル蛍光でカウントすることによって、25°Cから32°Cへの水温上昇が、褐虫藻の取り込みを低下させることを確認した。次に温度感受性の高いCS164株と同感受性の低いCCMP2459株を用いて、共生藻、宿主のいずれか、あるいは両者をあらかじめ25°Cあるいは32°Cの水温にそれぞれ暴露し、その後両者を共培養することによって感染効率の温度依存性に関する検証を行った。その結果、褐虫藻は温度感受性の高いCS164株のみが水温依存的に宿主への取り込みを減少し、CCMP2459株や宿主であるセイタカイソギンチャクの取り込みの温度依存的性は確認できなかった。この結果より、取り込み阻害は共生藻の温度感受性に大きく依存することが示唆された。同様の結果は、宿主動物に共生した状態の褐虫藻を高温処理した場合にも見られた。さらに、この取り込み阻害は褐虫藻の生存率、分裂速度、形態変化によるものではないことを確認した。また、共生藻と同サイズの蛍光マイクロビーズを共存させた取り込み実験から、CS164株が共存すると温度上昇によって触手への取り込みが顕著に減少していることが明らかとなった。この結果は、温度上昇によりCS164株と宿主の間で取り込みに必要な相互作用が影響を受けていることを示唆するものである。また、温度上昇によるCS164株の取り込み効率の低下は、5日間25°Cで培養する再活性化期間を設けることで、再び正常な取り込み率を獲得することが明らかとなった。しかし、この再活性化は、温度上昇期間が長くなると、起こりにくくなることも分かった。

次に、セイタカイソギンチャクやウスエダミドリイシ（サンゴ）を用い、高温ストレスによる褐虫藻の取り込み効率の低下が、白化や白化からの回復に影響することを示した。これらの研究成果は、刺胞動物と褐虫藻の共生は褐虫藻の温度感受性に大きく依存しており、褐虫藻は長期にわたる高温環境下では宿主個体から排出され、取り込みとの平衡が破綻することを明らかにしたものである。また、本研究は水温上昇によるサンゴの白化メカニズムの解明にもつながるものであり、審査委員会は、本論文が学位の授与にふさわしいと判断した。