Compound specific stable nitrogen isotope analysis of amino acids and sulfur-containing component analysis of reef corals: Toward method development for the prevention of coral bleaching and for recovering from damaged status.

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To save reef corals from bleaching event, we investigated possibility of enhancement of tolerance and resilience of corals against temperature stress by controlled feeding. By analyzing bulk stable carbon and nitrogen isotope ratios, as well as compound specific isotope analysis of amino acids of coral animal host and symbiotic algae under natural condition, we aim for enhancing our understanding of the major nutrition source and pathway, and for proposing coral bleaching vulnerability indicators for coral reef ecology. Sulfur containing protein and sulfate minerals were also analyzed by GC-MS and X-ray diffractometer. Although compound specific isotope analysis of amino acids is an established analytical method, accomplishing good derivatization of carboxyl and amine functional groups without generating incomplete derivatization or fragmental/recoupled impure molecules gain experience. Analytical machine side, internal/external leak, oxidation/reduction capability of reactors, surface area of catalyst, and degree of gas flow easiness inside of the reactor tubings are considered essential for stable and precise analytical campaign. Coral species used for this experiment (*Acropora hyacinthus* and *Acropora* cf. *glauca*) contain large number of cnidocytes, such as spirocysts and type b-mastigophores. To isolate symbionts from nematocysts, we applied flow cytometry technique with cell sorting function. Parameters, such as side scatter, forward scatter, green fluorescent, and chlorophyll autofluorescent induced by 488-nm blue laser emission were used for the isolation. By establishing those isolation and precise isotope analysis technique, we have developed evaluation roadmap to confirm an effect of nutrition control for the reef corals.

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