Application of Synchrotron Radiation-Based Infrared Microspectroscopy (SR-IMS) on Marine Animal-Plant Symbiosis

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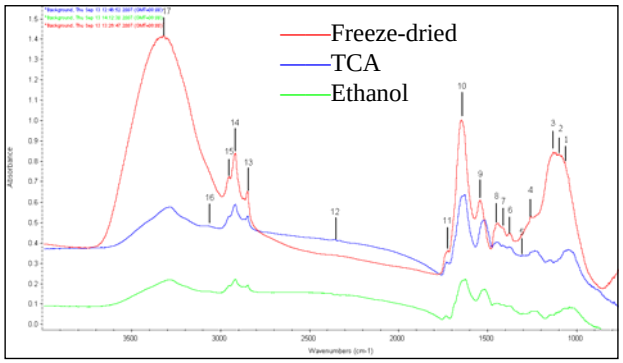
Endosymbiosis in cnidaria-dinoflagellate association plays critical role in regulating productivity of corals and related marine ecosystems. Cellular endosymbiosis with dinoflagellate *Symbiodinium spp*. (i.e. the symbiont or "zooxanthellae" in generic name) in corals (i.e. the host) is initiated by the internalization of symbionts via phagocytic process into host endoderm cells. However, the mechanism by which the symbiont is able to reside inside the host cell and establish an obligatory and mutualistic association remain unclear after four decades of investigation. Due to the large quantity of calcium carbonate skeleton, it is difficult to analyze how endosymbiosis is regulated and coordinated at organismal level. Fortunately, the non-destructive synchrotron-radiation-based microscopy in energy range of the infrared and hard X-ray provides a solution for investigating 2D images for chemical composition and 3D tomography images within coral tissue.

Based on the advantages of synchrotron-radiation-based microscopy, we will investigate the endosymbiotic association within coral tissue utilizing chemically resolved synchrotron-radiation-based infrared microspectroscopy (SR-IMS). By focusing on individual cellular activities as identified by SR-IMS, we propose to construct 2D chemical images for locating metabolite/key molecules and 3D tomography of endosymbiotic activity within coral tissue, and to learn how molecular and chemical organization in coral tissue can be correlated to endosymbiosis. We expect that this work will reveal new insights into the molecular mechanism of endosymbiosis in corals.

In this study, we applied the synchrotron radiation-based nano-transmission X-ray microscopy (SR-nTXM) to investigate the chemical composition of symbiotic and aposymbiotic dinoflagellate.



Annotation:
1. C-O stretch contributed from glycogen
2. $-PO_2^-$ symmetric stretch (DNA/RNA, dominant contribution , phospholipid and C-O stretch of glycogen)
3. C-O (?)
4. $-PO_2^-$ asymmetric stretch (DNA/RNA, dominant contribution and phospholipid)
5. nitro compounds (?)
6. CH3 bending (protein, dominant contribution, and lipid)
7. COO^- symmetric stretch (amino acid side chains, fatty acids) ?
8. CH_2 bending (lipid, dominant contribution, and protein)
9. amide II (protein N-H bend, C-N stretch)
10. Amide I (protrin C=O stretch)
11. Ester C=O stretch (Phospholipid or cholesterol esters)
12. CO_2 asymmetric stretch
13. $-CH_2$ symmetric stretch
14. $-CH_2$ asymmetric stretch
15. $-CH_3$ asymmetric stretch
16. Olefinic =C-H and N-H bending 1st overtone (amide B)
17. –NH stretching (amide A)

Figure 1. Effect of fixation on SR-IMS analysis of free-living dinoflagellate (*Symbiodinium* spp.). The free living (non-symbiosis) dinoflagellate (*Symbiodinium* spp.) samples were prepared with different fixative methods and plotted on low-E microscope slides ,respectively.

The preliminary data (Fig 1) demonstrated that the freeze-dried method gave the best results. Thus, we have established the suitable method for dinoflagellate sample. It also demonstrated that the synchrotron radiation-based nano-transmission X-ray microscopy (SR-nTXM) should be applied to investigate the endosymbiosis in cnidarian-dinoflagellate successfully.