Identification of Endosymbiosis-related Molecules in Cnidaria-dinoflagellate Association by Utilizing Synchrotron Radiation-based Infrared Microspectroscopy (SR-IMS)

Shao-En Peng (彭紹恩)[1,2], Huai-Ting Huang (黃懷葶)[1], Pei-Yu Huang (黃佩瑜)[3], Yao-Chang Lee (李耀昌)[3], and Chii-Shiarng Chen (陳啟祥)[1,2]

[1]Institute of Marine Biotechnology, National Dong Hwa University, Hualien, Taiwan
[2]Department of Planning and Research,
National Museum of Marine Biology and Aquarium, Pingtung, Taiwan
[3]National Synchrotron Radiation Research Center, Hsinchu, Taiwan

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.

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 detected and compared the chemical composition of symbiotic and aposymbiotic dinoflagellate by SR-IMS to explore the molecular mechanism of endosymbiosis.

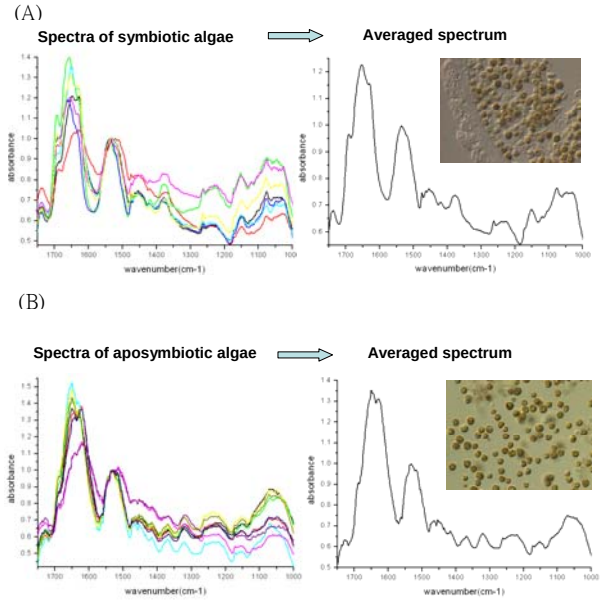
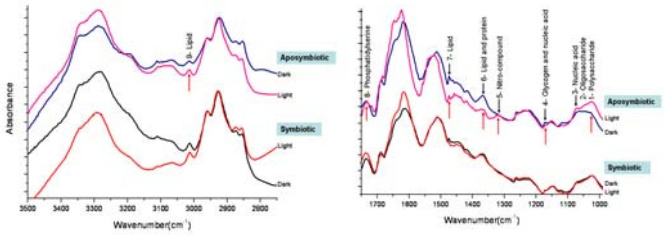


Fig. 1: IR spectra of (A) symbiotic and (B) aposymbiotic dinoflagellate (*Symbiodinium* spp.). The spectra are further averaged (~8 repeats) and normalized in the amide II band (1535 cm^{-1}). The image of symbiotic or aposymbiotic dinoflagellate also demonstrated.

We detected and obatined the spectra with ~ 8 repeats that prepared from symbiotic or aposymbiotic dinoflagellate (Fig. 1). Then, we compared the differenc of the spectra. According the observed spectrum, we interprete the possible biological molecules that contribute the specific spectum (Fig. 2).

The preliminary results of SR-IMS revealed that the chemical composition within dinoflatellate were different at symbiotic or aposymbiotic (free living) state, especially the glycogen, nitro-compound, lipid and phospholipids. These results also provided more evidences to demonstrate the specific regulation especially on lipid, glycogen, and nitrogen metabolism in cnidarian-dinoflagellate endosymbiosis.

(A)



(B)

No	Observed spectrum (cm^{-1})	Frequency range	Assignment	Biological molecule	Ratio of peak area: Symbiotic/ Aposymbiotic (Light)	Ratio of peak area: Light/Dark (Aposymbiotic)
1	1022	1160-970 1039-1021 1040-910	Polysaccharide backbones C–C symmetric stretching, CO symmetric stretching, C–OH bending P–O stretching	Polysaccharide Phosphorus compounds	2.2(↑)	0.9(–)
2	1045	1042 1049 1050	C–O stretching C-O stretching Symmetric P–O–H stretching	Oligosaccharides Glucoses Phosphorus compounds	1.15(–)	0.8(–)
3	1070	1080 1070	PO2- symmetric stretching CO-O-C asymmetric and symmetric stretching	Nucleic acids and phospholipids Lipids	1.16(–)	0.51(↓)
4	1170	1170 1170 1173 1160-1140 1260-1160	CO–O–C asymmetric stretching CO–O–C asymmetric stretching and symmetric stretching CO–O–C asymmetric stretching P–O stretching Asymmetric P–O–C stretching	Glucoses and nucleic acids Lipids Phosphorus compounds Phosphorus compounds	3.3(↑)	0.48(↓)
5	1322	1280-1400 1330-1290 1370-1300	55% NH in-plane bending, 19% CC stretching, 15% CN stretching , 11% CO in-plane bending 55% NH in-plane bending, 19% CC stretching, 15% CN stretching , 11% CO in-plane bending N=O stretching	Amide II Amide II Nitro-compounds	0.1(↓)	6.24(↑)
6	1370	1400-1370	COO symmetric stretching and CH_3 symmetric bending	Lipid and protein	2.6(↑)	0.26(↓)
7	1475	1471 1370-1450	CH_2 scissoring (methylic) N=O stretching	Lipid Nitro-compounds	0.97(–)	0.56(↓)
8	1733	1742-1720 1745-1720	C=O stretching C=O symmetric stretching	Phosphatidylserine Lipids	1.7(↑)	1.17(–)
9	3012	3012	Olefinic=CH stretching	Unsaturated lipid, cholesterol ester	4(↑)	0.9(–)

Fig. 2: Comparism of the IR spectra of symbiotic and aposymbiotic (free-living) dinoflagellate (*Symbiodinium* spp.). (A) the IR spectra of Free-living and freshly isolated dinoflagellate. (B) Tentative assignment of the diffenent bands frequently found in symbiotic or aposymbiotic dinoflagellate.