Symbiodinium sp. can stay alive through the gut and in faeces of cnidaria predators

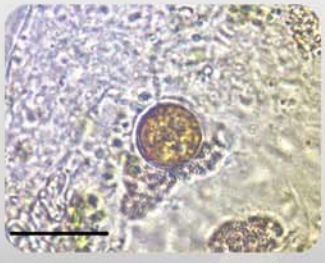
The case of *Coralliophilla meyendorffi* and *Anemonia viridis*

M. Poulicek [1], St. Roberty [1], S. Plaza [2], B. Gilbert [3], D.Fransolet [4] & O. Ladrière [1]

[1] Marine Ecology Unit (ULg, Belgium), [2] Oceanographic Station STARESO (Calvi, Corsica)
[3] Analytical Chemistry & Electrochemistry (ULg, Belgium), [4] Ecophysiology & Animal Physiology (ULg, Belgium)

Introduction

Many microalgae live in symbiosis with marine invertebrates. One of the most widely distributed and abundant intracellular algae are the "zooxanthellae" (dinoflagellates of the genus *Symbiodinium*) living symbiotically within cells of hermatypic corals and other cnidaria.
Many "strains" (now considered true species) were described, sometimes co-existing within the same host. Those "strains" exhibit different physiological properties leading to variable tolerance according to changes in environmental conditions (temperature, light, ...).The increased frequency of disruption of this symbiosis (coral bleaching) is a major threat for coral reefs future. In order to recover, bleached cnidaria can, either let proliferate the algae not previously expelled (5 to 25 % of the initial stock) or try to catch free *Symbiodinium* in the environment.Both processes potentially result in algae community changes, favouring environmentally better adapted strains ("adaptive" theory of bleaching).
As "free" zooxanthellae are quite rare in the plankton, one can look for other potential source of symbionts. Here we investigate the potential role of undigested algae embedded in the faeces of cnidaria predators as source of zooxanthellae for recolonization of bleached hosts



Cyst stage of a zooxanthella extracted from an *Anemonia viridis* tentacle (scale 10 µm)

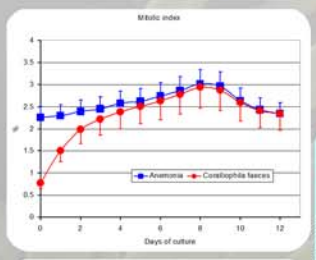
Motile zoospore (flagellated free stage) of a zooxanthella within the faeces of *Coralliophila meyendorffi* (Scale 10 µm)

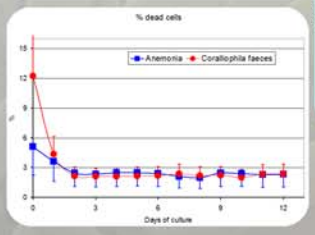
Material and Methods

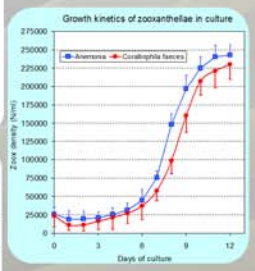
Anemonia viridis (Forskål)

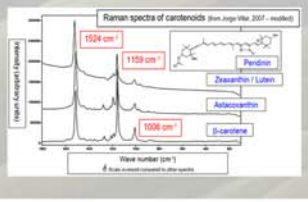
Coralliophila meyendorffi (Calcara)

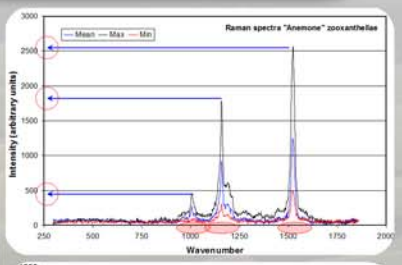
In June 2009, 15 live *Anemonia viridis* (Forskal) and 15 *Coralliophilla meyendorffi* (Calcara) were collected by scuba diving (3-8 m depth) and kept separate for acclimatization during one month in free flowing aquaria under in situ light and temperature conditions (18 to 22°C, max light intensity 1970 lux). Experiments were performed in July 2009.
"Anemone" zooxanthellae were collected by gentle homogeneization of 0.5 g (FW) tentacles in 10 ml of 0.2 µm filtered SW with a potter grinder, then purified from anemone debris by filtration (50 µm pore size) and centrifugation (500 x g), resuspended and cultured under in situ illumination and temperature in F/2 medium.
"Faeces" zooxanthellae were recovered by grinding freshly collected faeces of *Coralliophilla* exclusively fed with *Anemonia* for 1 week, then purified and cultivated the same way.
Algae density in culture was monitored in both cases for 12 days (5 replicates) by epifluorescence counts. **Mitotic indexes** (MI) were calculated (% of "doublets", N >500 cells) and **% dead cells** (Trypan blue permeation test, N>300 cells) in culture samples.
Apparition of motile (flagellated) cells was monitored along nycthemeral cycles (during the log phase of both cultures, days 6, 8 & 10).
Pigments change : **Laser Raman spectra** (incident light 514.532 nm) of individual zooxanthellae cells at different stages (cysts, ghosts, motile spore, ...) and of both conditions (freshly extracted from tentacles or isolated from faeces, without culture) were recorded (1 µm probe pointing on the chloroplast).

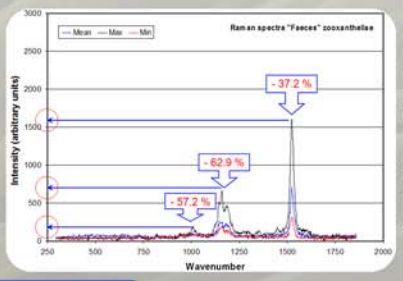


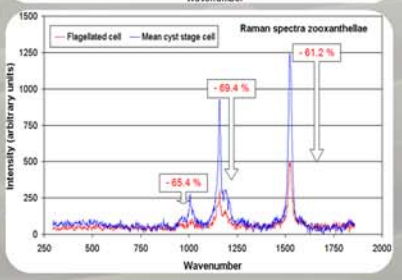


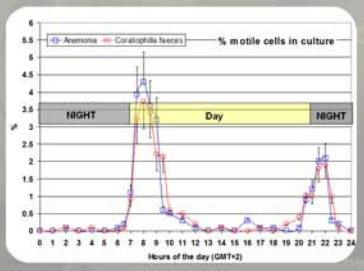












Results and discussion

- **Most zooxanthellae found in the faeces are alive (> 80 %) and able to multiply quickly.**
- Actively growing cultures were obtained from algae either directly extracted from anemones or from faeces. There are **no significant differences between the kinetics of both growth curves** : lag phase last approximately 6 days and logarithmic growth for another 6 days, culminating with densities around 200 to 250 103 algae ml-1 (inoculum was 25 103 cell ml-1).
- The MI of algae isolated from faeces is significantly lower than that of anemone extracted ones during the 3 first days of culture (0.8 to 1.2% compared to 2.0 to 2.8%) ; there is no significant difference during the following days. Maximum MI occurs after 8 days of culture (2.9 to 3.4%). Division activity occurs mainly during the very first hours of "light" period (from 6.30 to 9.00 AM, 200 to 600 lux) and at dusk (18h30 to 21h30, 400 to 50 lux).
- Division peaks were followed by **apparition of motile algae** (6h30 to 9h00 AM and from 20h30 to 22h00). There are virtually no flagellated forms during the night, and very few ones (< 0.2 % during the most illuminated hours of the day). Motile cells also arise from undisturbed fecal pellets but most flagellated spores cannot extract from mucous threads.
- % dead cells was higher just after isolation from faeces (10 to 25% of the population at day 1) compared to anemone extracted ones (2 to 7%). From day 3 to day 12, the % dead cells in culture remained constant around 2% of the algae population for both conditions.
- Raman spectra are characteristic of carotenoids (peridinin ?). These preliminary results apparently show a clear decrease of pigment response in faeces extracted cells compared to anemone isolated ones. Flagellated spores display the same pattern. Further research will show if this is due to a lower amount of pigment, masking or to a different molecular conformation.

Conclusions

- Most **symbiotic zooxanthellae** from Anemonia viridis consumed together with animal tissues by the gastropod Coralliophilla **withstand digestion** and are recovered alive, actively dividing and potentially motile in the faeces of the predator. This confirms results about predation of corals and anemones by tropical fishes (Arothron, Chaetodon, pomacentrids) and nudibranchs [Parker-Muller,1984]. A potential loss of carotenoid pigments is possible but has to be confirmed even if it was shown that "faeces zooxanthellae" were photosynthetically active.

- It has long been showed [Fit & Trench, 1983] that live Symbiodinium cells introduced in the coelenteron of cnidaria resist digestion and are internalized by endodermal cells. So, fecal pellets, once resuspended in the POM, could become a **source of algae for recolonization of bleached host**. Moreover, flagellated cells (motile spores) produced by undigested zooxanthellae in faeces are another potential source as these motile algae are attracted to potential hosts [Pasternak et al., 2006], if freed from mucous threads.

- It should be quite interesting to check the hypothesis that faeces and fecal pellets of cnidaria predators may constitute a reservoir of live zooxanthellae able to be consumed and internalized during recovering phases post-bleaching. This could contribute to redistribute algal strains and so **contribute to a higher genetic diversity** of "available" zooxanthellae as proposed in the **adaptive theory of bleaching** [Buddenmeier et al., 2004].

This study was supported by the ULg grant "Fonds Spéciaux" C-09/20, FNRS grant FRFC 2.4.631.09.F, and "Communauté Française de Belgique" ARC grant 05/10-33

More info : mpoulicek@ulg.ac.be, tel +32 (0)4 366 5067, www.leae.ulg.ac.be