CELLULAR MECHANISMS UNDERLYING BACTERIAL INFECTION PROCESS WITHIN THE LATERAL ZONE OF GILL FILAMENTS OF THE LUCINID *Codakia orbiculata*

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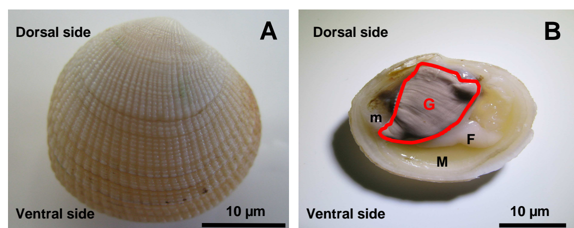
INTRODUCTION

Codakia orbiculata **is a shallow-water lucinid which inhabits low sulfide sediments in tropical seagrass *Thalassia testudinum*. The lateral zone of gill filaments of this species is the place of a chemoautotrophic symbiosis with sulfur-oxidizing bacteria located in specialized cells called bacteriocytes. This study was aimed at investigating the bacterial infection process in gills of decolonized individual. Two putative mechanisms have been suspected : cell division of internal bacteria and/or endocytosis process in which bacteriocytes absorb bacteria from the environment. Here, we attempt to evidence the mechanism that might underly bacterial infection processes during recolonization of gill filaments by using immunhistochemical, Card-FISH techniques associated with semi-thin sections.**

MATERIALS AND METHODS

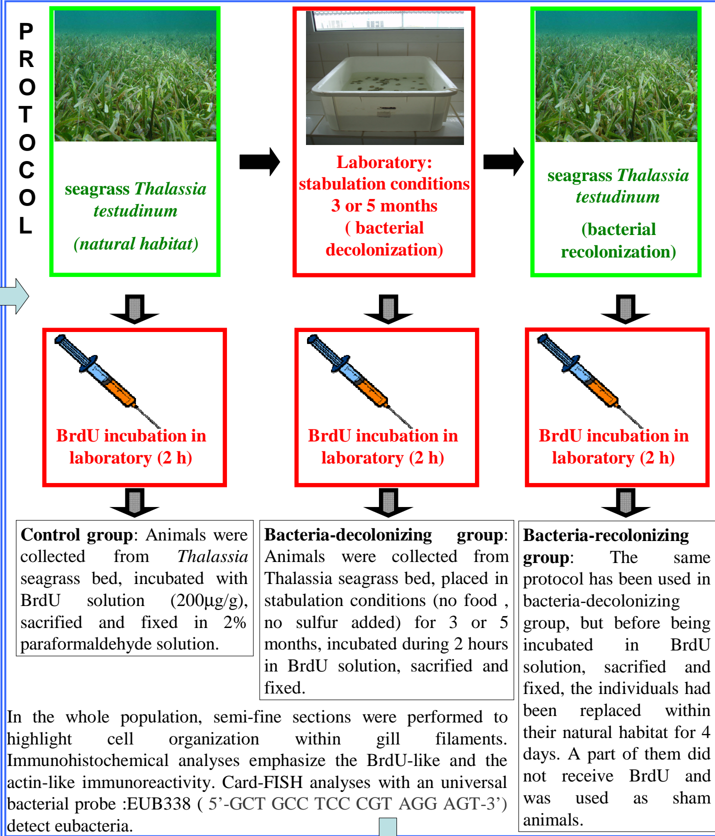
Biological material : *Codakia orbiculata* (Montagu, 1802)



Codakia orbiculata (A and B) was collected by hand from low-sulfide environment of *Thalassia testudinum* seagrass bed, in Guadeloupe (FWI, Carribean).

Macroscopic views of Codakia orbiculata

A: External view of the shell ; B: Anatomical view showing gills surrounded in red, foot (F), mantle (M), and adductor muscles (m).



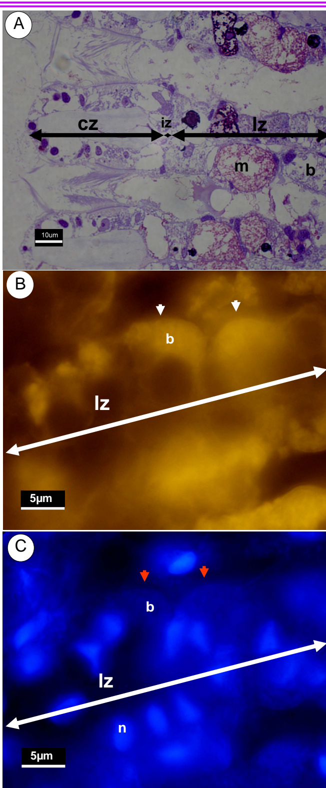
Control group: Animals were collected from *Thalassia* seagrass bed, incubated with BrdU solution (200µg/g), sacrified and fixed in 2% paraformaldehyde solution.

Bacteria-decolonizing group: Animals were collected from Thalassia seagrass bed, placed in stabulation conditions (no food , no sulfur added) for 3 or 5 months, incubated during 2 hours in BrdU solution, sacrified and fixed.

Bacteria-recolonizing group: The same protocol has been used in bacteria-decolonizing group, but before being incubated in BrdU solution, sacrified and fixed, the individuals had been replaced within their natural habitat for 4 days. A part of them did not receive BrdU and was used as sham animals.

In the whole population, semi-fine sections were performed to highlight cell organization within gill filaments. Immunohistochemical analyses emphasize the BrdU-like and the actin-like immunoreactivity. Card-FISH analyses with an universal bacterial probe :EUB338 (5'-GCT GCC TCC CGT AGG AGT-3') detect eubacteria.

RESULTS



Micrographs of representative gill sections obtained in 3 months starved animals recolonized 4 days. **Semi-thin section** (A) shows cell organization of the lateral zone of gills filaments including the ciliated zone (cz) (free zone of bacteria), the intermediary zone (iz) (composed of only one cellular layer), and the lateral zone (lz) (composed of 4 types of cells). Two of the latter cells are visible on micrograph: bacteriocytes (b) which contain bacteria and mucocytes (m). An Actin-labelling (white arrow heads) at apical poles of bacteriocytes observable with Alexa$_{456}$ with **immunohistochemical technique** (B). On the same section, blue fluorescence due to DAPI (C) shows eucaryote's DNA localised in the nucleus (n) and procaryote's DNA (red arrow heads) which is more diffuse. The actin-labelling is totally superimposable on bacterial localization suggesting a rearrangement of this protein at the site of entry of symbiosis-competent bacteria. This rearrangement may be due to phagocytosis which allows the entry of infectious microorganisms.

Micrographs of representative gill sections obtained in 5 months starved recolonized 4 days incubated in BrdU. **Card FISH technique** (D) illustrates the presence of bacteria in bacteriocytes revealed by FITC. Bacteria are only detected in the lateral zone, and are localized in bacteriocytes. With **immunohistochemical technique** (E), no BrdU positive bacteria have been detected. These observations allow to conclude that there's no division of bacteria in bacteriocytes at this delay.

BrdU-like immunoreactivity of control culture of bacteria (*Citrobacter sp.* gram negative).

Comparaison of bacterial DNA stained by DAPI (A,C) and after BrdU incorporation (B,D) from a log phase bacterial culture. *Citrobacter sp.* is a gram negative bacteria like the γ-proteobacteria gill-endosymbionts of *C. orbiculata*. Trying to detect procaryote's cell division, a bacterial log phase culture of *Citrobacter sp.* were incubated in BrdU at 200µg/ml during 2 hours (A,B). Approximately 100% of bacteria revealed with DAPI (A) were also labelled by BrdU (B) (green flurescence is due to FITC). In order to check that this labelling is due to BrdU incorporation, the same experience were done with a log phase culture of *Citrobacter sp.* non incubated in BrdU (C,D). In this case, while bacteria are positively stained with DAPI (C), there is no signal of fluorescence indicating that the labelling was likely due to the BrdU incorporation (D). According to theses results emphasizing gram negative bacterial division using BrdU at 200µg/ml is possible. This positive control allows to look at bacterial division in bacteriocytes.

CONCLUSION

No bacterial division was observed during the infection processes suggesting that this increase of bacterial endosymbiont is not due to the division of remaining bacteria but to the environmental acquisition of symbiosis-competent bacteria from the environment. The actin-like immunoreactivity of 3 months starved individuals confirms the hypothesis of an environmental transmission by phagocytosis, since a strong labelling, totally superimposable on bacterial localization, has been detected to the apical area of the bacteriocytes.