CELL INTERACTIONS INDUCED BY BACTERIAL INFECTION PROCESSES WITHIN THE LATERAL ZONE OF GILL FILAMENT OF THE LUCINID *Codakia orbiculata*

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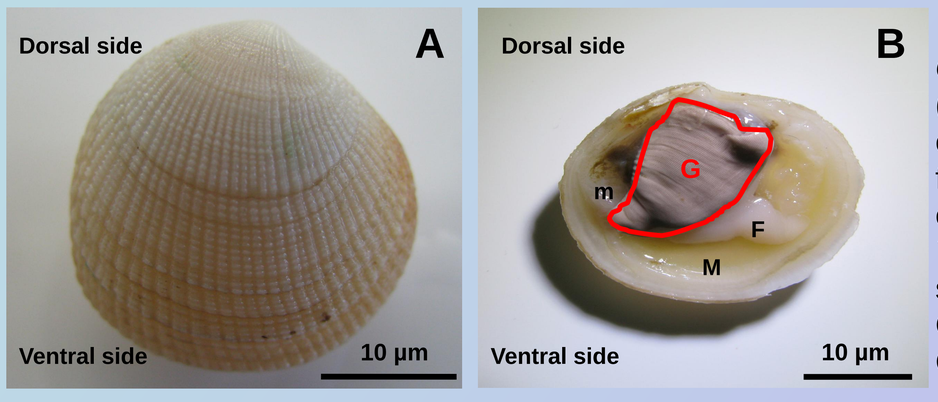
INTRODUCTION ***Codakia orbiculata*** **is a shallow-water lucinid which inhabits low sulfide sediments in tropical seagrass *Thalassia testudinum*. The lateral zone of gill filaments of this species is the place of a chemoautotrophic symbiosis with sulfur-oxidizing bacteria located in specialized cells called bacteriocytes. This study was aimed at investigating the bacteriocytes reorganization induced by bacterial infection processes. Two putative mechanisms of reorganizations for the bacteriocytes have been suspected : cell proliferation or variation of cytoplasmic volume. Here, we attempt to evidence the mechanism that might underly the adaptative plasticity of the bacteriocyte by using immunhistochemical and histological technics.**

MATERIALS AND METHODS

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



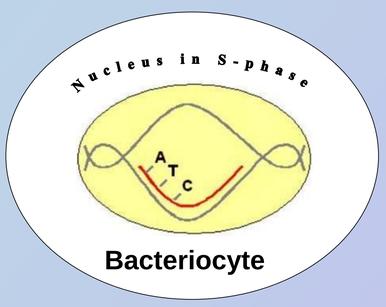
Codakia orbiculata (A and B) was collected by hand from low-sulfide environment from *Thalassia testudinum* seagrass bed, in Guadeloupe (FWI, Carribean).

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).

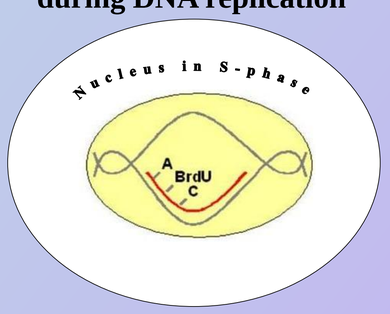
BrdU (5-bromodesoxyuridine) principle

normal replication

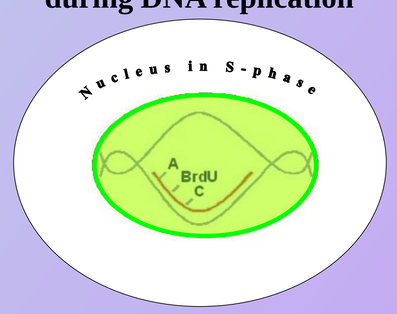


Undetectable mitotic cell by antibody anti-BrdU

BrdU incorporation during DNA replication



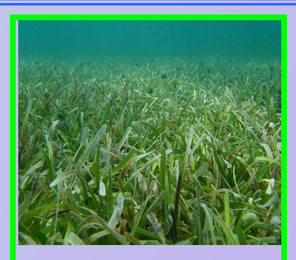
BrdU incorporation during DNA replication



Detectable mitotic cell by antibody anti-BrdU

BrdU is an analogue of thymidine which is incorporated in the DNA of proliferative cells during replication, and so is a marker of cell division.

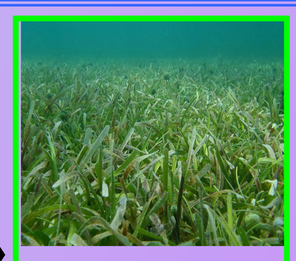
PROTOCOL



seagrass *Thalassia testudinum*

(natural habitat)

Laboratory: stabulation conditions 3 or 9 months (bacterial decolonization)



seagrass *Thalassia testudinum*

(bacterial recolonization)

BrdU incubation in laboratory (24 h)

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BrdU incubation in laboratory (24 h)

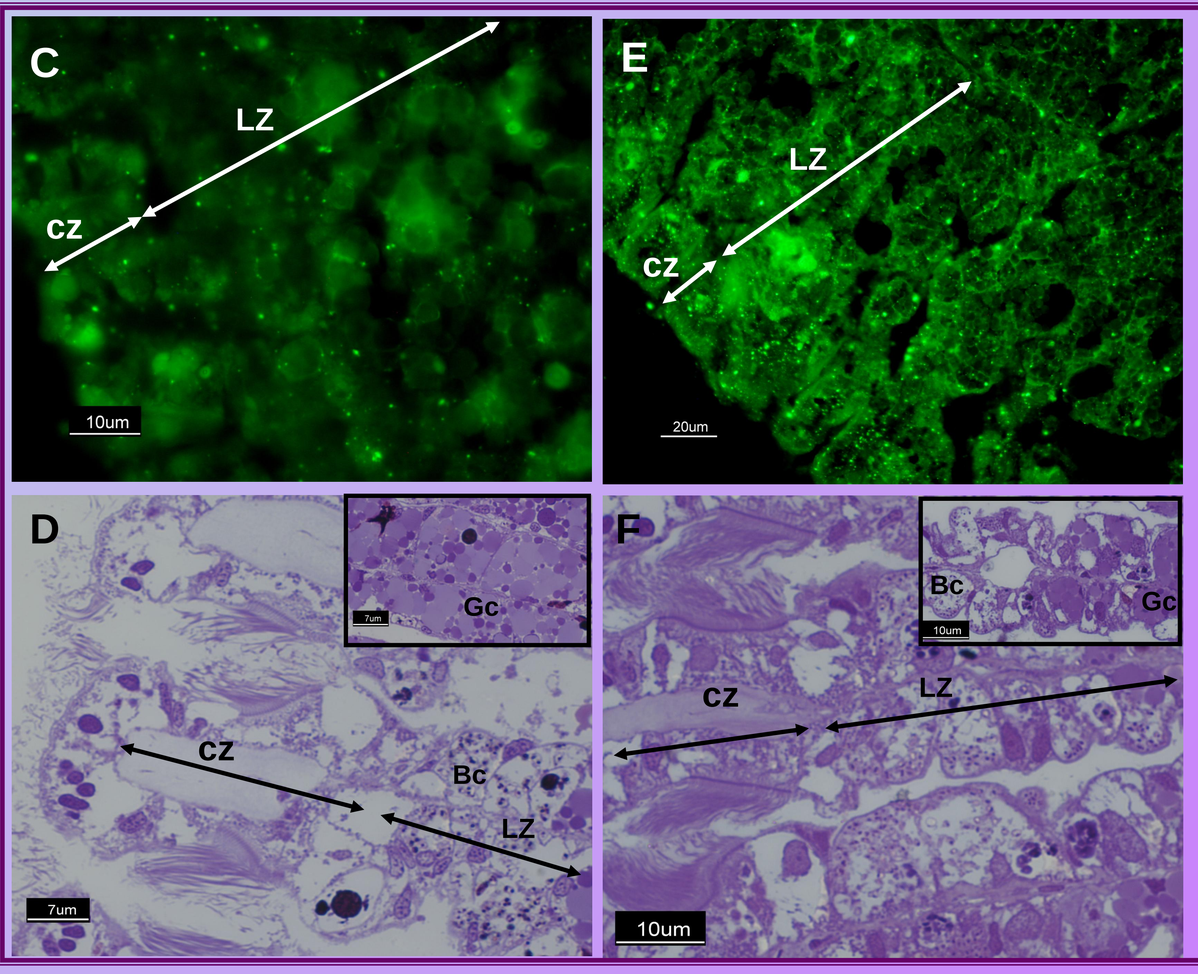
Control group: Animals were collected from Thalassia seagrass bed, incubated with BrdU solution (200µg/g), sacrified and fixed in 2% paraformaldehyde solution.

Bacteria-decolonizing group: Animals were collected from Thalassia seagrass bed, placed in stabulation conditions (no food , no sulfur added) for 3 or 9 months, incubated during 24 hours in BrdU solution, sacrified and fixed.

Bacteria-recolonizing group: Same protocol described used in bacteria-decolonizing group, but before beeing incubated in BrdU solution, sacrified and fixed, the individuals had been replaced within their natural habitat for 2 or 4 days.

In the whole population, immunohistochemical and histological technics were performed to evidenced respectively the BrdU-like immunoreactivity and cell organization within gill filaments.

RESULTS



BrdU-like immunoreactivity and cell organization in gill filaments.

Light micrographs of representative gill sections obtained in 5 months bateria-decolonizing group (C and D) and in 8 days bacteria-recolonizing group (E and F). ***Immunohistochemical technic (C and E)*** : illustration of the BrdU-labelling revealed by FITC (green fluorescence). On both micrographs, note no BrdU-positive nuclei have been detected. ***Histological technic (D and F)*** : illustration of cell organization of the lateral zone of gill filaments. Note that in lateral zone (inserts) the bacteriocytes (Bc) become the predominant cells in case of bacterial infection process in bacteria-recolonizing group (F) and non symbiotic granule cells (Gc) become the majority cells during bacterial decolonization process in bacteria-decolonizing group (D).

zc: ciliated zone; LZ: lateral zone; BC: bacteriocyte, b: bacteria

CONCLUSION **No BrdU-positive nuclei have been detected within the bacteriocyte of the lateral zone of gill filaments of the symbiotic model of *Codakia orbiculata*, while BrdU-labelling has been found in gill filaments of *Brachidontes exastus* used as positive controls to validate the BrdU technic. Taken together, these results indicate there's no cell division of the bacteriocytes within bacteria-decolonizing or bacteria-recolonizing roups at the examined delays. The variation of bacteriocyte organization during bacterial infection process is not due to a variation of the number of bacteriocytes, but likely to a change in cytoplasmic volume of these cells. During bacterial decolonization, the cytoplasmic volume may decrease with progressive loss of bacteria, whereas during bacterial recolonization, the bacteriocyte may increase its cytoplasmic volume in order to fill up with bacteria.**