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Béziat Naéma, Sébastien Duperron, Olivier Gros. Characterization of bacterial ectosymbionts colonizing gills and endophragm of two crabs: *Aratus pisonii* and *Uca rapax*. Caribbean Science and Innovation Meeting 2019, Oct 2019, Pointe-à-Pitre (Guadeloupe), France. hal-02423817

HAL Id: hal-02423817

<https://hal.univ-antilles.fr/hal-02423817>

Submitted on 7 Jul 2020

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CHARACTERIZATION OF BACTERIAL ECTOSYMBIONTS COLONIZING GILLS AND ENDOPHRAGM OF TWO CRABS: *ARATUS PISONII* AND *MINUCA RAPAX*.

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Here we present the first description of interactions between bacterial ectosymbionts and two mangrove crabs: *Aratus pisonii* (Sesarmidae) and *Minuca rapax* (Ocypodidae). These crabs belong to the order Decapoda and to the infra-order Brachyura known as "real crab". Specimens were collected in Guadeloupe on the mangrove trees *Rhizophora mangle* for *A. pisonii* and from the mangrove mud for *M. rapax*. To observe ectosymbionts on Electron Microscopy (Scanning and Transmission, SEM and TEM), gills were fixed in a 2.5% glutaraldehyde solution in 0.8x PBS buffer for 24h at 4°C. Then they were rinsed in the same buffer and dehydrated in graded concentration of acetone. Finally, they were critical point dried and sputter coated with gold before observation on a Quanta 250 SEM. For TEM observations, gills were fixed in 2.5% glutaraldehyde in 0.1M cacodylate buffer for 45 minutes at RT in the dark. Then, they were rinsed in the same buffer then smaller pieces of gills were fixed, for 1 h in 1% osmium tetroxide in the same buffer before a post-fixation with 2% aqueous uranyl acetate for 1 hour at RT. Finally, they were rinsed in H₂O and dehydrated in ethanol before embedding in LR White resin. A couple of ultrathin sections (60nm thick) were observed for each crab species analyzed by using a FEI Tecnai G20 at 200 kV.

SEM and TEM observations showed that ectosymbionts colonize gills and endophragm (axial skeleton related to legs) for all individuals investigated (Figure 1). For both species studied, several different bacterial morphotypes (cocci, rods, but no filaments) were observed throughout the surface of gill discs and endophragm according to SEM views (Figure 1A).

Symbionts did not cover the entire surface of gill discs. They formed patches irregularly distributed while they formed a uniform bacterial biofilm which covers the entire endophragm (data not shown). Moreover, no intracellular bacteria could be observed according to TEM views (Figure 1B).

Whole DNA was extracted from gills and PCR using universal primers to amplify the V3-V4 region of the 16S rRNA-encoding gene were performed. The PCR products sequences have been analysed confirming that several bacterial species are involved per crab species. Five main bacterial species are detected for the two crabs' species. However, the bacterial community composition is totally different between *Aratus pisonii* and *Minuca rapax*. Moreover, the proportion of the main bacterial species involved in this symbiotic relationship varies according to individuals within the same crab species. Such bacteria belong to Alpha-proteobacteria, Actinobacteria, Flavobacteria, and Bacteroidetes phyla. Most of the bacteria involved represent either a new genus or a new species based on the sequence of a widely accepted marker gene, namely 16S rRNA.

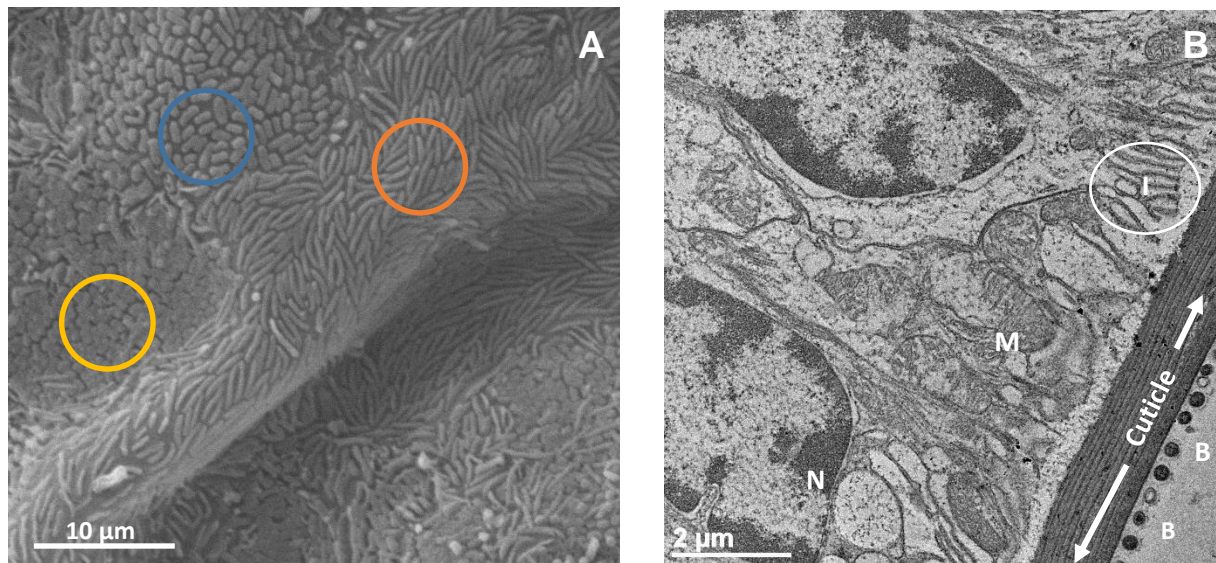


Figure 1: Ultrastructural analysis of gill tissue from *Aratus pisonii* and *Minuca rapax* disc gills.

A: SEM view of the surface of a disc gill from *A. pisonii* where different bacterial morphotypes are observable (circles yellow, orange and blue). Such diversity was confirmed by phylogenetical analysis.

B: TEM view of a transverse section of *M. rapax* gill disc. The cuticle is thick on this species, no intracellular bacteria were observed within the cytoplasm of the gill cells. Only one layer of bacterial ectosymbionts is observed (B: bacteria, M: Mitochondria, N: Nucleus, I: Ionophore).

Such interactions between terrestrial crustaceans and bacteria can play many roles. In deep-sea hydrothermal vent shrimps, bacterial ectosymbionts established on the cuticle of the branchial chamber realize carbon-fixation and transfer it to the host [1]. Environmental parameters (as pH, sulfide concentration, etc.) can be important factors that can influence the diversity of the bacterial community involved but also the role of such symbiotic community [2]. In this case, it's hard to compare hydrothermal organism's role to mangrove organism's role because they live in chemically different environments, and the bacterial ectosymbiont community structure is also different. On hydrothermal crustaceans, filamentous bacteria are often observed [3] while on mangrove crabs, only rod-shaped bacteria were observed. The difference can be related to their environment, it would be interesting to know what relationship is established between host and symbionts in this mangrove ecosystem.

Further investigations will improve the identification of the bacterial ectosymbiont identity, inform the distribution on the gill filaments and on the endophragm as well as putative metabolic interactions between the eukaryotic host and its bacterial partners in order to give some clues on the nature of this relationship. This will define more accurately the nature of the relationship, and its potential benefits to each of the partners. We will also check if the bacterial symbionts are present in the crab's environment, in order to define the transmission mode of bacterial symbionts to the next crab generation and during molt events.

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