Bacterial symbiosis in the bivalve *Cardita calyculata* (L., 1758)?

Juan Diego Castro-Claros*[1], Begoña González-Ruiz[1] and Carmen Salas[1]

*Corresponding author: juandiegocastroc@gmail.com
[1]Departamento de Biología Animal, Universidad de Málaga, Campus de Teatinos s/n, 29071- Málaga (Spain).

Keywords: Bacteria, Biofilm, *Cardita calyculata*, Mollusca, Symbiosis

Introduction

Bacteria are one of the most frequent group in symbiotic relationships with invertebrates, as ectosymbionts (Duperron et al., 2008) or as endosymbionts (Moran and Baumann, 2000). Some symbiotic bacteria seem to be related to biomineralization processes (Braithwaite et al., 2000), but most of them seem to be related to food resources. *Cardita calyculata* (L., 1758) belongs to the ancestral clade Archiheterodonta, in which the presence of bacteria in symbiosis has been observed in other families. So the observation of bacteria on the shell of some specimens of *C. calyculata* raised the question whether this presence is circumstantial or if it is related to its habitat or its phylogenetic position.

Materials and methods

Specimens of different sizes were collected in the intertidal and upper sublittoral zone of the Mijas coast (Málaga), under stones buried in the sediment and between the algae of the upper fissures. These specimens were transported to the laboratory in seawater to be relaxed with magnesium chloride. Later, several of them were fixed in 4% formaldehyde for paraffin inclusion and others in glutaraldehide (2.5% in PBS) for TEM analysis. Some blocks of female specimens with developed gonads were deparaffinized and critical point dried to be seen in the SEM (Scanning Electron Microscopy, JEOL JSM840). The shells were observed in a FESEM (Field Emission Scanning Electron Microscope, Zeis Auriga Cross-Beam Station). The specimens for TEM (Transmission electron microscopy, JEOL JEM-1400) were treated with 1% osmium tetroxide and 2% uranyl acetate to provide contrast to the samples, were included in resin (EMbed 812 of EMS) and ultrathin sections (50-70 nm) were made with an ultramicrotome (Leica Ultracut R). The bacteria from the biofilm present on the shells were cultured in a marine nutrient broth (CULTIMED) at 37°C for 24h. After having made dilutions, was sown on marine nutritive agar (CULTIMED), in order to obtain pure cultures. Later, these bacteria were subjected to Gram staining (observation to optical microcopy) and to Negative staining (observation to TEM).

Results and discussion

A bacterial biofilm was observed on the shell of all specimens analyzed, regardless of the type of habitat (under stone or in algae), bathymetry (intertidal or sublittoral) or size (ultra-juvenile, juvenile and adult). Among the bacterial types we observed different types of bacilli, including coccobacilli, cocci and spirochetes, (Figure 1). However, the shells living under stones in the intertidal showed a greater diversity and density of bacteria than the shells living in the intertidal algae.

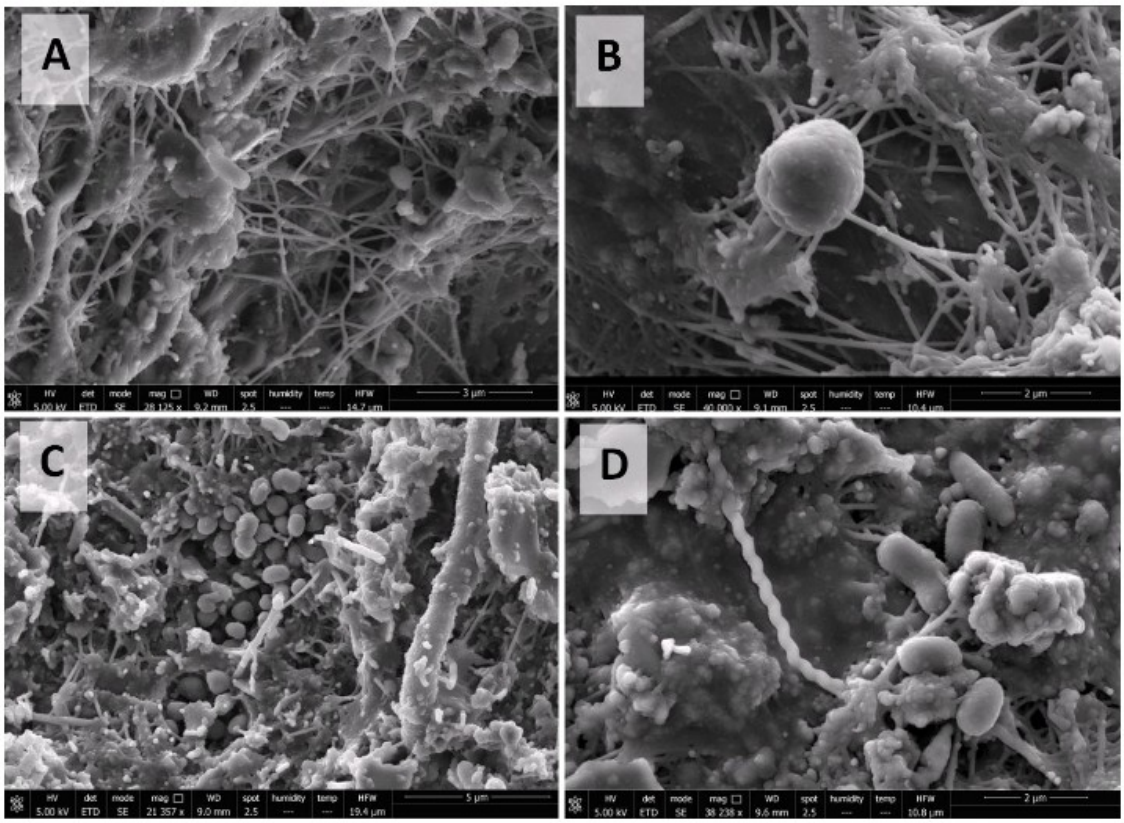

Figure 1: Bacterial biofilm on the shell of the *Cardita calyculata*, observed in FESEM. A. Biofilm produce by bacteria. B. Bacterium type coccus producing biofilm. C. Cocci, coccobacilli and bacilli. D. Spirochete.

From the culture of bacteria we obtained gram-negative bacilli and cocci (Figure 2)

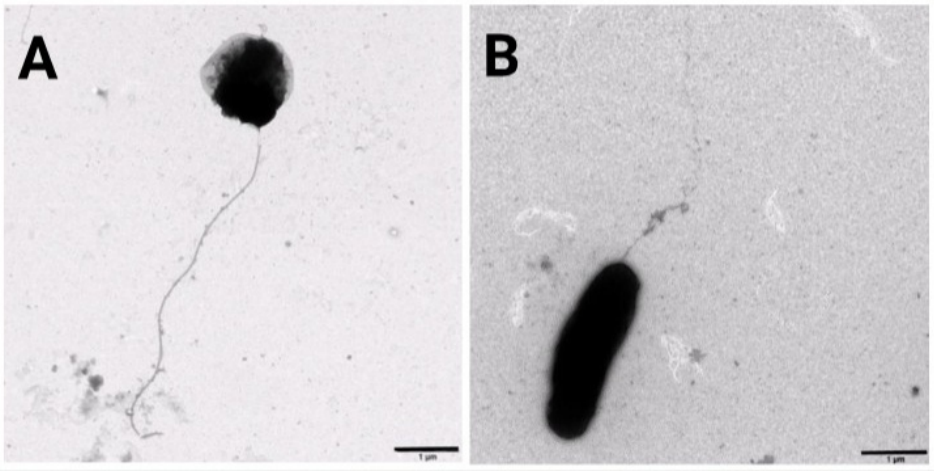

Figure 2: Bacteria from the culture, subjected to Negative staining and observed in TEM. A. Coccus with a flagellum. B. Bacillus with a flagellum.

The analysis of the soft parts of the bivalve allows us to see the presence of bacteria inside the female gonad, on the surface of the oocytes (Figure 3). Also, the presence of bacteria inside bacteriocyte was observed in the mantle edge.

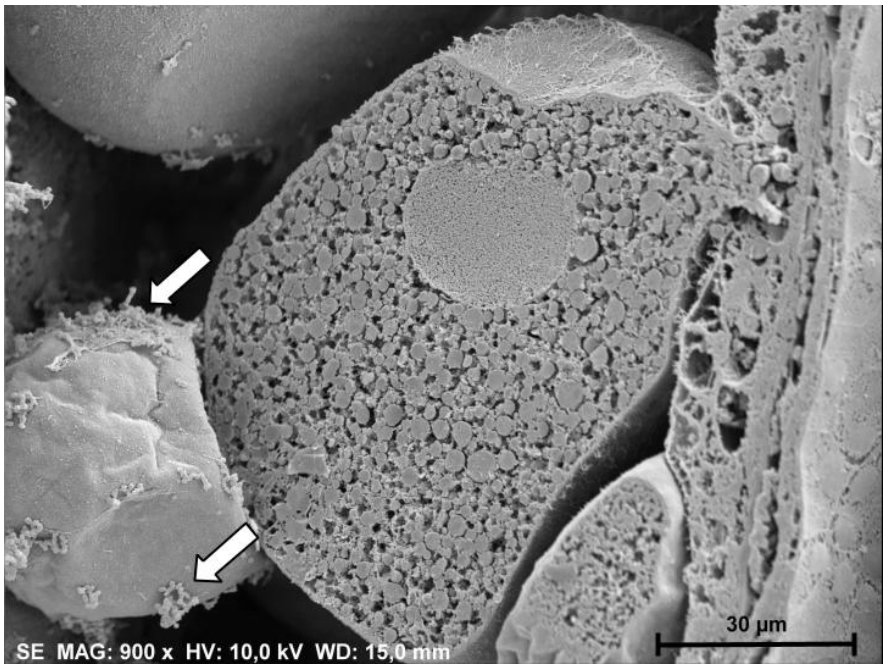

Figure 3: Bacteria inside the female gonad, on the surface of the oocytes, observed in SEM (arrows).

The presence of a bacterial biofilm on the shell surface of all specimens, regardless of habitat or age, seems to indicate that it must be an adaptive selection for the species, since the juveniles settle initially in the algae, but subsequently migrate to cavities of the rocks or under stones. The greater diversity and bacterial density in the specimens living under stones could be related to the decrease of oxygen and the redox potential of these environments, as has been shown in *Montacuta ferruginosa*, species that lives in reducing environments and as well as *C. calyculata* also presents bacterial biofilm (Gillan et al., 1998). On the other hand, the presence of bacteria inside the gonad, on the oocytes, would indicate a vertical transmission of the bacteria from parents to offspring (Bright and Bulgheresi, 2010), independently of that, can be also collected from the environment. The vertical transmission of bacteria in bivalves is considered as an indicator of symbiosis between both organisms.

References

Braithwaite, C.J., Taylor, J.D., Glover, E.A., 2000. Marine Carbonate Cements, Biofilms, Biomineralization, and Skeletogeneis: Some Bivalves Do It All. Journal of Sedimentary Research 70, 1129–1138.

Bright, M., Bulgheresi, S., 2010. A complex journey: transmission of microbial symbionts. Nature Reviews Microbiology 8, 218–230.

Duperron, S., Halary, S., Lorion, J., Sibuet, M., Gaill, F., 2008. Unexpected co-occurrence of six bacterial symbionts in the gills of the cold seep mussel *Idas* sp. (Bivalvia: Mytilidae). Environmental Microbiology 10, 433–445.

Gillan, D.C., Speksnijder, A.G., Zwart, G., De Ridder, C., 1998. Genetic Diversity of the Biofilm Covering *Montacuta ferruginosa* (Mollusca, Bivalvia) as Evaluated by Denaturing Gradient Gel Electrophoresis Analysis and Cloning of PCR-Amplified Gene Fragments Coding for 16S rRNA. Applied and Environmental Microbiology 64, 3464–3472.

Moran, N.A., Baumann, P., 2000. Bacterial endosymbionts in animals. Current Opinion in Microbiology 3, 270–275.