ADHESION OF BACTERIA AND DIATOMS TO THE EXOSKELETON OF THE HARPACTICOID COPEPOD Tigriopus fulvus IN CULTURE: ELECTRON AND EPIFLUORESCENT MICROSCOPE STUDY

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INTRODUCTION

In marine environments microrganisms can survive as free forms or bound to animate and inanimate surfaces (1,2). The microbial colonization of an organic substratum is a very complex phenomenon; the initial event in biofilm formation is the collision (random collision or chemotaxis) between microrganisms and surface. This is followed by the adhesion of picoplankton to the organic substratum (3).

Chitin is one of the most abundant organic compounds in nature and it consists of ß-(1,4) linked N-acetylglucosamine units; in marine environments several tons of chitin are annually produced by copepods (4). These crustacea represent a significant percentage of zooplankton communities (5-8) and they are heavily colonized by bacteria, mainly Vibrios (9,10), that are responsible for the mineralization of this insoluble polysaccharide by specific enzymes (11,12). This process is essential for recycling carbon and nitrogen in marine ecosystems (13).

The association between epibiotic microrganisms and plankton is extremely advantageous. In fact adhered cells can survive in seawater longer than free forms, as a strategy for survival in stressful conditions (14). With regard to this, it has been suggested that epibiotic bacteria may be optimally positioned to exploit organic (amino acids) and inorganic (NH_4^+) nutrients released by planktonic copepods (15).

The present study has investigated the biofouling of Tigriopus fulvus in culture. In addition, this paper also describes possible consequences of

the microbial colonization on human health and on the lifestyle of this copepod.

MATERIALS AND METHODS

T. fulvus was caught from the rockpools of Genoa-Nervi (Ligurian Sea) with sterile bottles and placed in seawater previously filtered through fiberglass filters and membrane filters (Gelman GN/6, 0.45 (m), in thermostat bath (25°C), with constant conditions of salinity (‰) and pH (8.0-8.2). Animals were fed on marine microalgae Tetraselmis suecica Kylin (Butch) commonly used in aquaculture (16) and kept in a culture medium (Walne solution, sterile seawater and vitamin solution). They were maintained in a thermostatic chamber (Haeraeus BK 6160) and bubbled with filtered air at 14°C constant temperature; illumination was provided by four cool-white fluorescent tubes and a 12:12 h-light:dark cycle was used (16).

Some of these copepods were prepared for observation under scanning electron microscope (SEM). Crustacean zooplankton was rinsed five times (3 min each time) carefully in sterile seawater and then fixed with 4% buffered seawater formalin. The copepods were dehydrated in ethyl alcohol gradients, dried in a critical-point drying apparatus and coated with gold in a ion-spattering apparatus.

Other copepods were collected and investigated by means of epifluorescence microscopy. They were suspended in sterile seawater, fixed as previously described and stained with 4',6-diamidino-2-phenylindole (DAPI, Sigma) at a final concentration of 0.5 μ/ml for 7 minutes. Finally copepods were collected onto 25 mm black polycarbonate Nucleopore membranes with a 0.2 μm pore size (Sigma). Slides were observed on an epifluorescent Olympus microscope IX-FLA inverted reflected light at 40x magnification.

RESULTS

Preliminarily, to evaluate associations between picoplankton and T. fulvus, copepods were observed by means of epifluorescence microscopy. Then SEM was used to study more closely the microrganisms which coated

these animals.

Fig. 1 shows several colonies of bacteria stained with DAPI and located on the dorsal surface of T. fulvus. Red autofluorescent algae were observed; they were dispersed over copepod skeleton, especially appendages and caudal region (fig. 2).

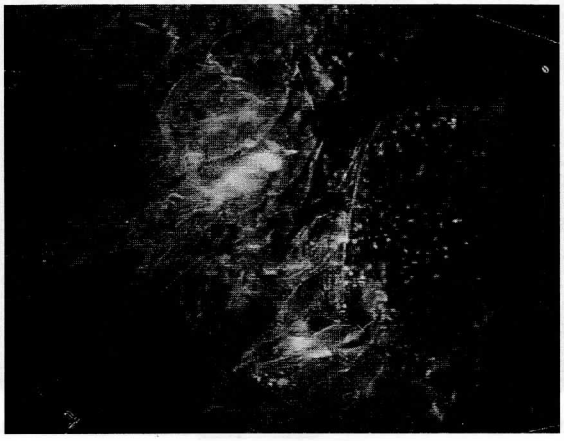

Fig. 1 - Adult T. fulvus stained with DAPI: epifluorescence photograph showing colonies of bacteria on the dorsal side of the copeod (40x).

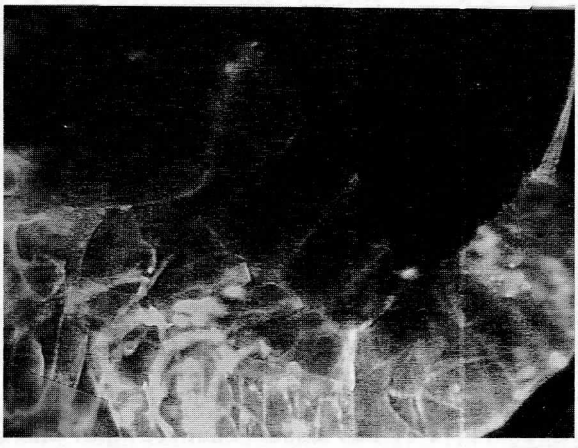

Fig. 2 - Adult T. fulvus: red autofluorescence algae are seen (40x).

Analysis by SEM revealed high concentrations of bacteria mainly located on the ventro-lateral side (fig. 3).

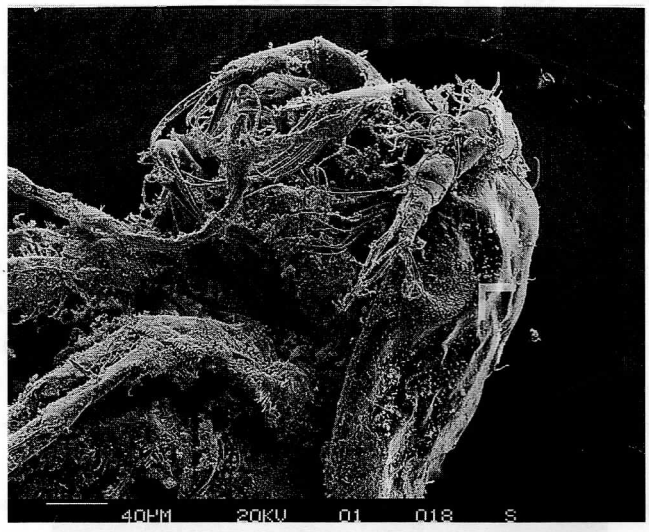

Fig. 3 - Bacteria associated with T. fulvus and observed under scanning electron microscope. An arrow indicates numerous bacterial cells located on the lateral side of copepod skeleton.

Adhesion to copepods was selective, since the heaviest concentrations of bacteria were observed on the appendages (fig. 3), joints of segments (fig. 4), and oviger eggs (fig. 5).
Numerous diatoms, probably belonging to the genus Cocconeis, were seen; they were found on bacterial biofilms (fig. 4).

DISCUSSION

Epibionts of T. fulvus consists of bacteria and algae; bacterial cells probably belong to the genus Vibrio, although the species were not identified by SEM and epifluorescence microscopy. In fact, it has been showed that in marine environments the major biofoulant of T. fulvus is Vibrio algynoliticus (17). It has been observed that the exoskeleton of Acartia clausii, which is a dominant species in Tokyo Bay, is heavily

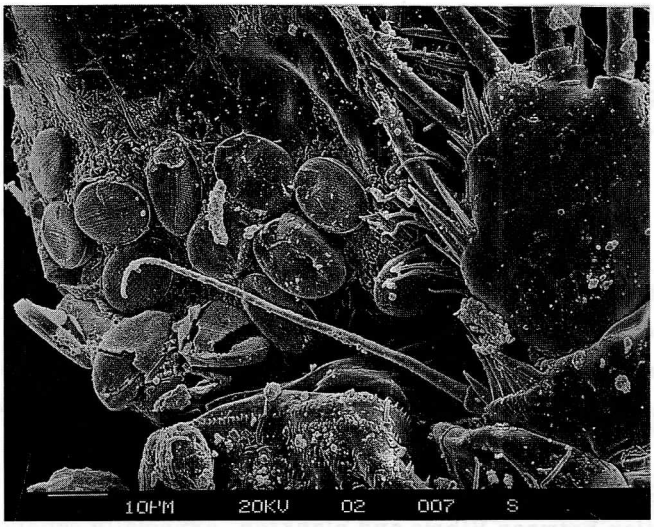

Fig. 4 - Adult female T. fulvus observed under scanning electron microscope: high magnification of ventral region showing diatoms and bacteria associated with copepod.

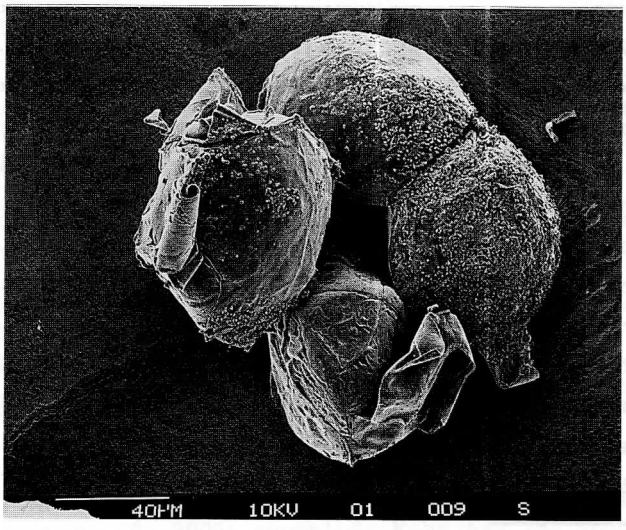

Fig. 5 - Oviger eggs of adult female T. fulvus: scanning electron photograph showing high concentrations of bacteria on the surface of egg sac.

colonized by bacterial cells, probably Vibrio spp. (18). These microrganisms are located on the joints of segments, legs, swimming legs themselves and depressed part of the body; moreover, the adhesion of epibiotic cells has been observed more frequently on the ventro-lateral side than on the dorsal side (18). Hansen and Bech (19) have also found that the surfaces of Acartia tonsa in culture are abundantly colonized by bacteria (expecially Vibrio spp.).

It has been showed that Vibrio algynoliticus has evolved specific proteins to recognize and associate with chitin and the efficiency of attachment is dependent on NaCl concentration and temperature (20). This microrganism has been found on the body of other zooplanktonic organisms. For example, it has been observed that the exoskeletons of Artemia nauplii were colonized by this bacterium (21).

The association between Vibrio and plankton, expecially copepods, is very important; in fact, several studies show that crustacean zooplankton may serve as reservoir of pathogenic bacteria, including Vibrio cholerae (22-24). It has also been observed that adhered vibrios can enter a viable but non culturable state when conditions are not favorable; under specific conditions, VBNC cells can revert to their vegetative state, i.e., actively growing state (9,14,22). Moreover, the ability of these microrganisms to stick to plankton allows them to be transmitted to organisms grown in aquaculture systems which use live plankton as feed (21). Bacteria produce extracellular polymers which can support adhesion of algae, mainly diatoms (25). These microrganisms, in their turn, synthetize molecules which stimulate bacterial metabolism and feed on substances produced by bacteria themselves (25).

In conclusion these data support previous suggestions (22,26) that high concentrations of microrganisms on the exoskeleton of T. fulvus could affect processes such as reproduction or feeding through interactions between chitin, adhered bacteria and diatoms.

In the marine environment associations between picoplankton and copepods are very important not only for the lifestyle of these animals,

but also for marine ecosystems and human health. In the present study we investigated the biofouling of Tigriopus fulvus in culture and possible effects of this colonization by means of scanning electron microscopy and epifluorescence microscopy. These investigations show that T. fulvus is heavily and not uniformly colonized by bacteria and algae.

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