EPIBIOTIC BACTERIA IN THE MARINE ECOSYSTEM

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The colonization of a substratum by microbes is a survival strategy that allows them to adapt to the variations of environmental conditions and to get nutritive substances. In fact, all marine substrata, including the planktonic organisms, can easily be colonized by bacteria. In particular, planktonic copepods can act as carriers to promote microbial spreading and vibrios were observed to adhere frequently to gut and external surface of copepods. In this connection, the colonization of chitin exoskeleton of the copepod Tigriopus fulvus by vibrios was studied. Microbial population adhering to copepods was observed to be mainly characterized by Vibrio alginolyticus, a pathogenic species for humans. The adhesion of vibrios varied with environmental conditions (mainly with water temperature and NaCl concentration); furthermore, these bacteria were found on copepods also when they were not onbserved free in water. V. alginolyticus can also synthesize proteins useful for the adhesion to chitin; hence, four binding chitin polypeptides (molecular weight 14-53 Kda) were identied by electrophoresis (SDS-PAGE). These results confirm the role of the zooplankton as a reservoir for spreading of pathogenic and non pathogenic bacteria in the marine ecosystem.

Organic matter in marine ecosystems is essentially produced by picoplankton and phytoplankton. The organic matter so synthesized passes in the food web where it is assimilated by primary consumers and transferred to subsequent links. In the chain of detritus organic matter

is demolished and decomposed by bacteria and protists up to complete mineralization. Among the energy transfer mechanisms occurring within the planktonic biocenosys a very important collateral circuit to the classical food web phytoplankton-zooplankton-fish, is namely "microbial loop", where fluxes occur only among bacteria, phytoplankton and protists and nutrients are rapidly remineralized (fig. 1) (1).

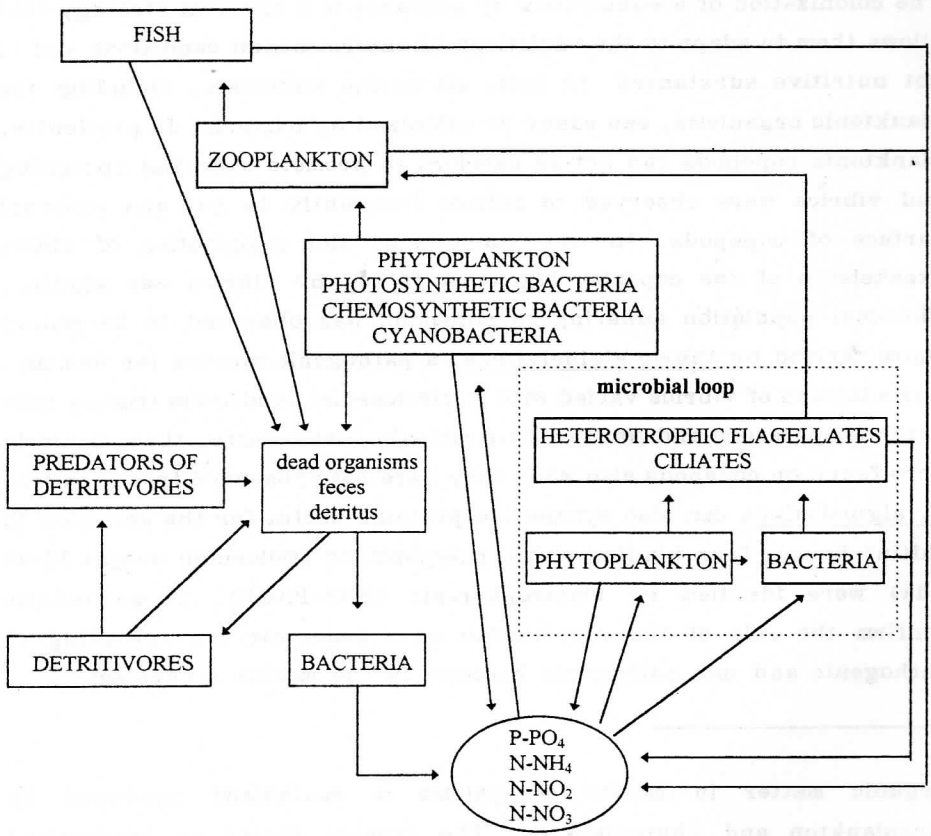

Fig. 1 - Relationships between microbial loop and aquatic food web.

Recently the importance of particulate organic matter weakness in marine ecosystems (2) and in laboratory controlled conditions (3) was emphasized. It was also observed that the availability of organic matter resistant to decomposition can be higher when it is colonized by microbial communities (4).

Furthermore, the colonization of a substratum is a good survival strategy, as it allows microbes to adapt themselves more easily to variations of environmental conditions, and also allows them to get their required nutritive substances with major efficiency (5). This phenomenon is highly complex and involves changes of microbial morphology, density and metabolism (6).

All marine substrata can be colonized by bacteria; when the target is a planktonic organism the colonization is a useful mechanism to increase spreading of microbes (7,8). Indeed, several studies have emphasized that planktonic organisms, besides constituting an environment suitable for bacterial growth, also act as carriers in order to promote their displacement to zones having nutrient concentrations and light conditions useful to support growth and survival (5).

In this connection, it was recently observed that planktonic copepods are carriers suitable to promote microbial spreading (7-9); for this reason, they have been taken as research model for the study of bacteria-zooplankton association (8,10,11). This has been mainly due to both the wide diffusion of these crustaceans within the plankton, where they sometimes exceed 70% of total population, and to the easy rearing of some species.

Particularly, vibrios were observed to be the most frequent bacteria adhering to the copepod external surface and to gut; it is noteworthy that within the genus Vibrio some species pathogenic to humans are included (8,12).

Besides, epibiotic organisms were demonstrated to colonize definite parts to exploit excretion of dissolved organic compounds and inorganic nitrogen produced by hosts (13).

Considering the importance of this subject within the marine ecosystem,

the colonization by vibrios of chitin exoskeleton of the copepod Tigriopus fulvus living in Ligurian rock pools was firstly examined. During the last decades this copepod was extensively studied as regards morphology, reproduction, development and influence of environmental factors on its population (14-17).

Microbial population adhering to copepods is mainly characterized by Vibrio alginolyticus (fig. 2) (18), heterotrophic bacterium known to be a pathogenic species for humans (18). In particular, the adhesion of vibrios increases when environmental conditions are favourable for their growth; furthermore, vibrios were recorded adhering to copepods even if they were not observed free in water.

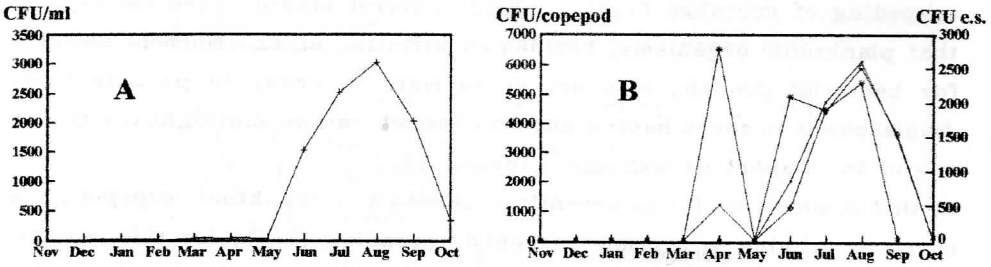

Fig. 2 - Total counts of viable vibrios, both free in rock pool water (A) and associated with T. fulvus (B). Symbols: (●) male copepod; (◆) female copepod; (*) egg sac (e.s.).

It was also observed that the exoskeleton of copepods, composed by chitin a resistant to degradation compound, is a substratum suitable for adhesion and growth of V. alginolyticus. In this connection, it was observed that adhesion highly depends on water temperature and NaCl concentration (in vitro the bulk of bacteria was recorded at 20°C and 3% of NaCl) (19). It was also demonstrated that V. alginolyticus is able to synthesize proteins useful for adhesion to chitin (fig. 3) (19); four polypeptides with molecular weight included between 14 e 53 Kda, able to bind chitin, were identified by electrophoretic methods (SDS-PAGE).

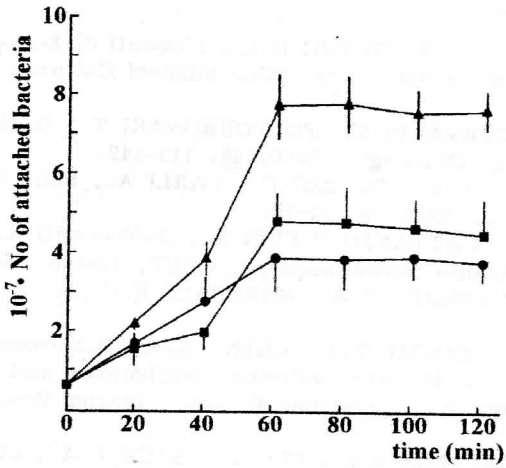

Fig. 3 - Time course of Vibrio alginolyticus T3 attachment to chitin particles. Results are means of three experiments performed with three cultures on different days; bars represent SD. Similar results were obtained with strain P2. The original bacterial inoculum was $5x10^7$ (●), $1x10^8$ (■) and $3x10^8$ (▲).

These proteins were recorded both in bacteria isolated from exoskeleton of copepods and in vibrios free in water; their expression does not seem to depend on the occurrence of chitin, chitobiose and chitotriose in culture medium (19).

V. alginolyticus was isolated also from the zooplankton collected along the coast of Senigallia (Ancona, Adriatic Sea) between May 1996 and April 1997; it was the sole easily isolable species from plankton >200 μm sized; otherwise, from plankton sized between 0.45 μm and 200 μm both Vibrio alginolyticus (between June and September and also in March) and Vibrio vulnificus (between April and May and in March) were separated (20). According to previously reported data (20), such results confirmed the role of zooplankton as a natural reservoir for spreading of bacteria, including species of interest in human pathology, in the marine ecosystem.

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