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Ultrastructural observations of the reaction of *Chondrilla nucula* (Porifera, Demospongiae) to bacterial invasion during degenerative processes

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Abstract : Exogenous bacteria are able to penetrate into the *Chondrilla nucula* body during degenerative processes. The morphology of these microorganisms, their production of spores and their alterations due to the sponge reaction have been studied.

Résumé : Pendant les processus de dégénérescence chez *Chondrilla nucula,* on a observé une population de bactéries exogènes capables de pénétrer dans l'éponge. On a étudié la morphologie de ces organismes, la production des spores et leurs altérations liées à la réaction de l'éponge.

INTRODUCTION

The presence of extracellular bacteria is a common phenomenon in the mesohyl of Porifera (Sarà & Vacelet, 1973). Such microorganisms may even occupy, as in *Aplysina,* 38% of the tissue volume (Bertrand & Vacelet, 1971; Vacelet, 1975), thus constituting a nutritional source on which the various cell types can feed either directly by active phagocytosis (Vacelet, 1971, 1975, 1979 ; Gaino *et al,* 1977) or indirectly as a result of the degeneration of bacteria (Sarà *et al,* 1973).

The association seems to be more closely related to the sponge organization and to the development of the aquiferous system, than to ecological conditions or systematic position (Lévi & Lévi, 1965 ; Vacelet & Donadey, 1977 ; Wilkinson, 1978 a).

The variety of morphological types of microflora associated with Porifera, as shown by means of ultrastructural investigations, has raised the question of a possible specificity of these symbionts. Microorganisms with deeply modified cell walls have been described and, such a morphology can be considered as an adaptation connected with endosymbiotic life (Vacelet, 1975 ; Vacelet & Donadey, 1977 ; Wilkinson, 1978 a).

Moreover, the host sponge seems to be able to control its associated population, by affecting bacterial organization, reproduction and growth rate. The association is so strict in *Apfysina* that, with the first degenerative processes, the disappearance of the typical bacteria and their replacement with exogenous ones have been observed (Bertrand & Vacelet, 1971 ; Vacelet, 1975) ; these latter should be related to sponge degeneration (Vacelet, 1979).

This phenomenon has not been deeply investigated and studies on the symbiosis between bacteria and sponges have mainly dwelt upon the morphological aspect, with some attempts at classification by isolating bacterial strains selected from some sponge species (Bertrand & Vacelet, 1971 ; Wilkinson, 1978 b ; Wilkinson *et al*, 1981).

As the microflora is very abundant in *Chondrilla nucula* mesohyl (Gaino *et al*, 1976, 1977), this species has been utilised to investigate the reaction against exogenous bacteria which occurs during degenerative processes resulting from protracted laboratory culture.

MATERIAL AND METHODS

The specimens were collected along the coast of Portofino promontory, Italy, at a depth of nearly 10 m and kept in a well-oxygenated, cooled aquarium (18° C constant temperature).

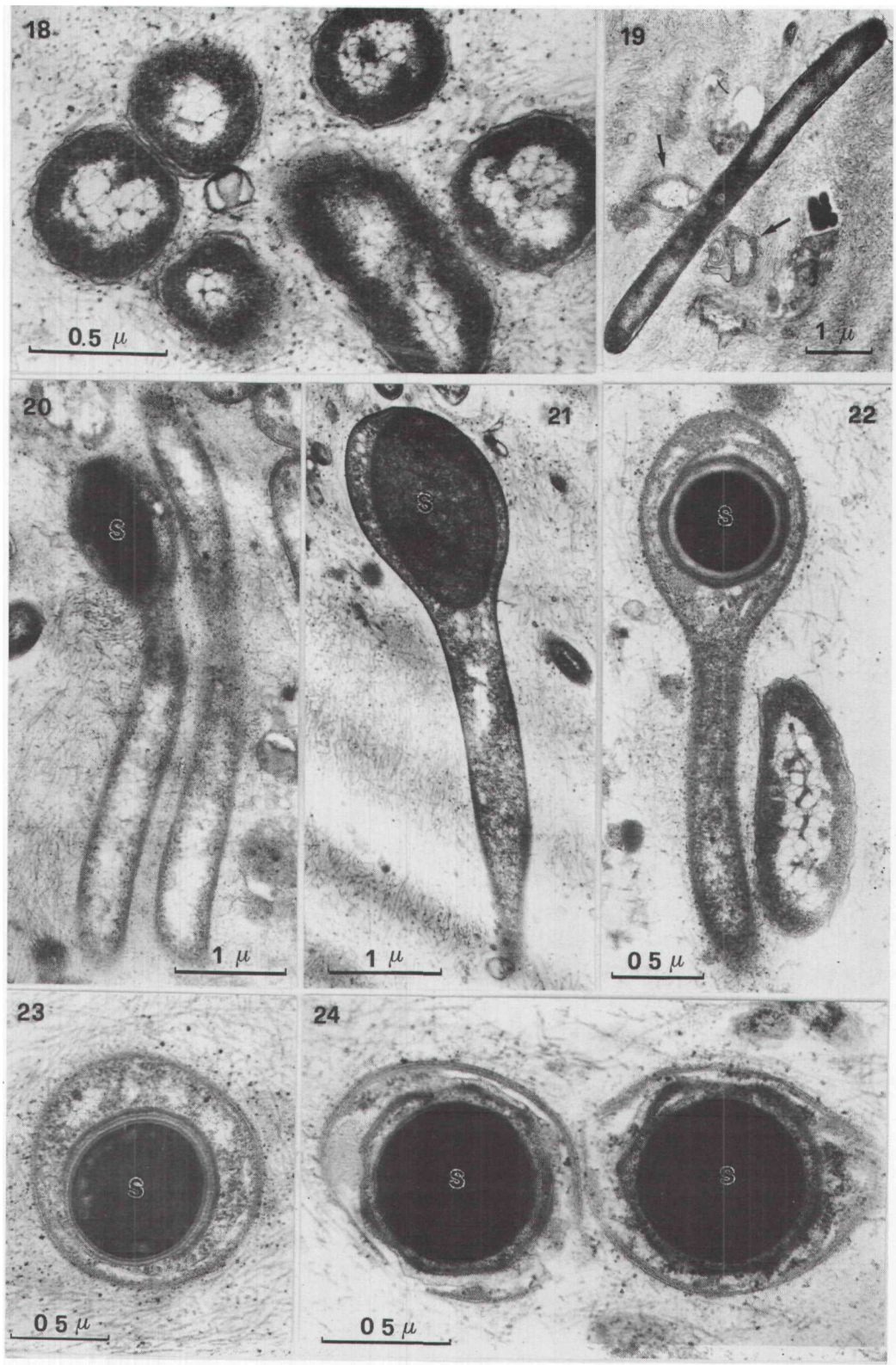
Nearly a month later, when an evident bacterial film could be observed on the sponge surface, some specimens were fixed in 2.5 % glutaraldehyde in artificial sea water. After rinsing in sea water with addition of 6.5 g/1 of NaCl (Vacelet, 1975) and postfixing for 1 hr in 2 % osmium tetroxide in sea water, the specimens were dehydrated in alcohol and propilene oxide and embedded in Araldite. Thin sections were stained with uranyl acetate and lead citrate to improve contrast. Thick sections for light microscopy were stained in toluidine blue.

The thin sections were observed on a Zeiss EM 9 electron microscope.

RESULTS

Observations were carried out on the ectosome and the choanosome.

Fig. 1 - Thin section of superficial ectosome with high concentration of exogenous bacteria (→). SC : spherulous cells.
Fig. 2 - Part of the sponge framed in figure 1, with dense bacteria on the surface.
Fig. 3 - Exogenous bacteria which have penetrated the ectosome.
Fig. 4 - The same exogenous bacteria, characterized by a reticulate nucleoplasm, in transverse and longitudinal section.
Fig. S - Collagen fibrils (→) beside an exogenous bacterium which has penetraded the ectosome.
Fig. 6 - Thin section tangential to the external surface of the sponge. The darker areas represent zones with higher bacterial concentration. SP : spicules.
Fig. 7 - Dense zone of bacteria in the framed zone of figure 6.
Fig. 8 - Morphology of exogenous bacteria that have penetrated into the mesohyl. The fibrillar component of the collagen is irregular and loose.
Fig. 9 - Undulating external wall and endocellular vacuolarization in some bacteria.
Figs 10-11 -Exogenous bacteria with an elongated shape in the most internal strata of the ectosome. The collagenous fibrils are less frequent around the bacteria.



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several direct attempts to compare the bacterial population of a sponge body with that living in the environment (Madri *et al*, 1971; Wilkinson, 1978 b), have not been resolved ; 2) ultrastructural studies directed to define the different morphological types (Vacelet, 1975 ; Vacelet & Donadey, 1977 ; Wilkinson, 1978 c) did not explain till now the origin of a matrix bacteria population.

Also the degenerative processes seem to be caused by the action of bacteria, even if these phenomena differ from case to case. In *Aplysina cavernicola*, for instance, the beginning of decomposition and the appearance of bacteria normally absent from the host tissues (Bertrand & Vacelet, 1971) showed that the sponge is able to choose its bacterial population and to defend itself, in normal conditions, against the penetration of elements which could damage it.

In *Chondrosia reniformis*, whose resistance to degenerative processes is well known (Nardo, 1847), it has been observed that fragments separated from the sponge body show an active proliferation of symbiotic bacteria and that the decomposition of the fibril bundles is related to the collagenolytic activity of these bacteria (Garrone, 1975, 1978).

It has been observed that in normal conditions *Chondrilla nucula* does not show any antimicrobial activity (Burkholder & Ruetzler, 1969). It is though possible that the sponge operates its defense mechanisms when interested by a massive bacterial invasion.

Some investigations of the marine bacteria and of the bacteria from different sponge species, among which *Chondrilla nucula*, have shown an *in vitro* degeneration of the collagen that is only due to the specific bacteria of the sponge (Wilkinson *et al*, 1979).

In *Chondrilla nucula* tested in our investigation, loosening of the superficial collagen fibers helps the penetration of exogenous bacteria that form the outer bacterial film covering the sponge.

Observing the sponge it has been noticed the absence, in the ectosome stratum, of the bacteria normally associated with the sponge tissue in *Chondrilla nucula* (Gaino *et al*, 1977), while it has been found the presence of elements constituting the external bacterial film. The penetration of such bacteria is probably due to both the loosing of the ectosome collagen texture and to the active action on the fibrils of the bacteria themselves.

Such a possibility seems unlikely, however, since, after penetrating into the sponge, the bacteria mass only in the most superficial strata.

In some exogenous bacteria in the sponge it is possible to observe a number of endocellular vacuoles that could represent cellular alterations due to the new environment in which the bacteria find themselves. The absence of choanocyte chambers in the choanosome of the sponge indicates the absence of an active aquiferous system.

This phenomenon has been observed also in disturbed areas of *Aplysina cavernicola* (Vacelet & Gallissian, 1978). It is possible, then, that with the beginning of

degenerative processes, the bacteria, which have penetrated into the sponge body through the canals of the aquiferous system, are able to invade the sponge body. Bacteria with a reticulated nucleoplasm, typical of those found in the superficial zone of the sponge have arrived in the most internal parts of *Chondrilla nucula* through the canals. The alteration in the bacteria cells wall could represent a reaction of the host tissue to these microorganisms.

Degenerating bacteria characterized by the presence of internal fibrils can also be observed in the variously fragmented collagen. The production of spores from some lengthened bacteria, the morphology of which is modified by the sporulation processes, could also be connected to a reaction of the host tissue.

As sporogenous bacteria are usually anaerobic, the absence in *Chondrilla nucula* of choanocyte chambers and hence of water currents in the sponge could provide favorable conditions for development of the bacteria. Since the spores are produced by the bacteria as forms of resistance, their presence in the bacteria inside the sponge could signify that the sponge is attempting a defence against the bacterial invasion. Such a reaction is underlined by the fact that in some of the sporogenous bacteria the outer coat of the spores appears deeply altered.

As the degeneration of bacteria and spores is common also in the zones still in good condition, it is possible that such a process may be the result of a defensive reaction by *Chondrilla nucula*. This phenomenon takes place even if wide areas of the sponge body are damaged as well as marked by the invasion of ciliates.

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