ACTA GEOLOGICA HISPANICA, v. 37 (2002), nº 1, p. 53-60

Endolithic Microorganisms in Live and Dead Thalli of Coralline Red Algae (Corallinales, Rhodophyta) in the Northern Adriatic Sea

L. A. GHIRARDELLI (1)

(1) Dipartimento di Biologia, Università degli Studi di Trieste, via L. Giorgieri 10, I-34127 Trieste (Italy). E-mail: ghirardl@univ.trieste.it

ABSTRACT

Live and dead thalli of crustose coralline algae were examined to evidentiate their endolithic flora. As it occurs in corals, there is a great difference between endolithic microorganisms observed in live thalli and those observed in dead thalli.

During our study live thalli were found to have few (*Plectonema terebrans* and *Ostreobium quekettii*) or no endolithic microorganisms, whereas a more numerous number of microorganisms (cyanobacteria, chlorophyta and fungi) was found in dead thalli.

Keywords: Endolithic microorganisms. Bioerosion. Coralline algae. Cyanobacteria. Chlorophyta. Fungi. Borings.

RESUMEN

Se examinaron tallos vivos y muertos de algas coralinas crustosas para evidenciar su flora endolítica. Como sucede con los corales, existe una gran diferencia entre los microorganismos endolíticos observados en tallos vivientes y los observados en tallos muertos.

Durante el presente estudio se ha encontrado que los tallos vivos tienen pocos (*Plectonema terebrans* y *Ostreobium quekettii*) o ningún microorganismo, mientras que en los tallos muertos se observó un mayor número de ellos (cianobacterias, clorófitas y hongos).

Palabras clave: Microrganismos endolíticos. Bioerosión. Algas coralinas. Cianobacterias. Clorófitas. Hongos. Perforaciones.

INTRODUCTION

The bioerosion of corals, in particular *Porites lobata*, by micro- and macroborers was widely studied by several authors (Le Campion-Alsumard et al., 1995; Peyrot-Clausade et al., 1992; Chazotte et al., 1995), as coral skeletons are the most common substrate for endoliths (*sensu* Golubic et al., 1981) on coral reefs.

The chlorophyte *Ostreobium quekettii* BORNET and FLAHAULT (Le Campion-Alsumard et al., 1995), together with small fungal hyphae (Kendrick et al., 1982; Bak and Laane, 1987), was identified to be the major endolithic constituent of live corals. Other endolithic microorganisms, such as the cyanophyte *Plectonema terebrans* BORNET and FLAHAULT, play a subordinate role (Laborel and Le Campion-Alsumard, 1979). In addition to *Ostreobium quekettii* and *Plectonema terebrans* (Lukas, 1974), conchocelis stages of the red alga *Porphyra* (Laborel and Le Campion-Alsumard, 1979) were observed in live corals.

On the contrary a more diverse endolithic microflora was described from skeletons of dead corals: *Gomontia, Mastigocoleus, Plectonema* and *Hyella*, in addition to *Ostreobium*, are the most common inhabitants (Weber-Van Bosse, 1932). The species composition of euendolithic microflora was similar to that encountered in other carbonate substrates.

Crustose coralline algae also represent one of the most important biological and geological components of tropical coral reef ecosystem. These algae cement together sand, dead corals and debris to create a stable substrate (Littler and Littler, 1995). Crustose coralline algae, particularly *Hydrolithon onkodes* (HEYDRICH) PENROSE and WOELKERLING is the principal cementing agent that produces the structural integrity and resilience of the outer reef rim.

Studies carried out on *Hydrolithon onkodes* on the French Polynesian reef (Tribollet and Payri, 2001), revealed that the microborers form a green band composed by *Ostreobium quekettii* and *Plectonema terebrans.*

Few other studies were carried out on microendoliths boring crustose coralline algae. A small sheeted cyanophyte, identified as *Plectonema terebrans*, occurs in the cell wall of the live cells of the coralline algae *Titanoderma pustulatum* (LAMOUROUX) NÄEGELI and *Lithophyllum incrustans* PHILIPPI, collected in the gulf of Trieste (Italy) (Ghirardelli, 1998).

During this research one of the problems was to understand whether the algal thallus was dead or alive, because, as the coralline algae grow apically, the upper zone is usually alive, whereas the basal part of the thallus is dead.

Previous works tried to correlate the colour of crustose coralline algae with their photosynthetic activity: red thalli can be considered alive, while grey or white thalli are usually dead (Nichetto and Ghirardelli, 1992).

MATERIALS AND METHODS

Specimens of calcareous red algae: *Lithophyllum incrustans* PHILIPPI, *Hydrolithon farinosum* (LAMOUROUX) PENROSE and CHAMBERLAIN and *Titanoderma pustulatum* (LAMOUROX) NÄEGELI - were collected in the littoral settings of Trieste (Northern Adriatic Sea, Italy) at 1-2 metres depth. They were usually still anchored to little stones representing their substratum. The coralline algae were then removed from the substratum in laboratory.

The live and dead parts of algae were then treated for SEM, TEM and light microscope observation and to produce resin casts.

Scanning Electron Microscope (SEM): The specimen was fixed with 4% glutaraldehyde in 0.6 M phosphate buffer, rinsed in distilled water, dehydrated in graded alcohol series, critical point dried, mounted on a stub and golden coated. Specimens were then observed with a Leica Stereoscan 430i.

Resin casts: The specimens were fixed as described above, rinsed in distilled water, dehydrated by transferring specimens through a series of acetone solutions in distilled water, along a gradient of increasing concentration, ending with several baths in pure acetone. The infiltration phase began with a 50:50 solution of ARALDITE mixture (Durcupan ACM) and acetone. Specimens were then infiltrated with pure Araldite mixture and polymerised at 60° C for 48 hours. The blocks were cut open and the substrate was etched using Perenyi solution (0.5% chromic acid, 10% nitric acid, 70 to 90 % alcohol, in relation 3:4:3). Resin casts were golden coated and then observed by SEM (Golubic et al., 1970).

Transmission Electron Microscope (TEM): In order to eliminate the carbonate deposition from the cell wall, decalcification was achieved by treating it in a fixative (4% GTA phosphate buffered solution) containing 5% EDTA, which was changed frequently, for 24 hours. Specimens thicker than one mm were decalcified, with Perenyi solution for some hours, before the fixation in 4% GTA phosphate buffered solution. After washes in buffer, the specimens were postfixed in 1% osmium tetroxide in the same buffer, washed in water, dehydrated in a graded acetone series and embedded in Spurr resin (Spurr, 1969). Sections were stained with uranil

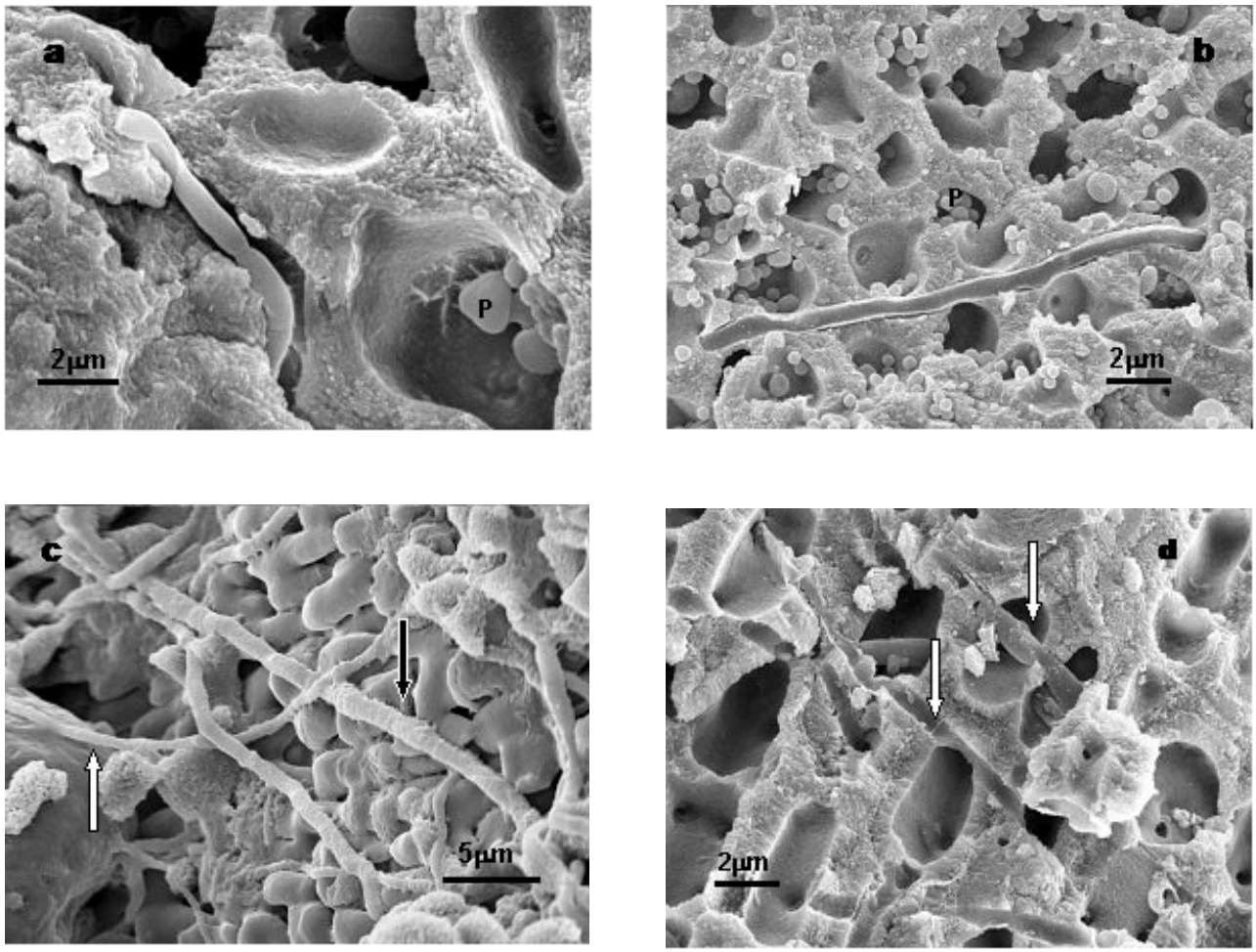

Figure 1. SEM photographs of endoliths in live and dead thalli of *Lithophyllum incrustans*. (a) *Plectonema terebrans* in a live tissue of *Lithophyllum incrustans*; Plastids (P) are visible. (b) Sheet of a cyanophyta, boring the cell wall of a live tissue; (P) = Plastids. Resin cast of microendoliths in live tissue of a coralline alga. (c) Black arrow indicate the resin cast of the tunnel of *Ostreobium*, while the white arrow indicate the resin cast of the tunnel of *Plectonema.* (d) Fungi in a dead thallus; arrows indicate that fungi are able to bore the calcified cell walls and to cross the internal of the cells.

acetate and lead citrate (Reynolds, 1963) and observed with a Philips EM 201.

Light microscope: Before decalcification, the epilithic algal turf grown on coralline algae was removed by sodium hypochlorite and by a vigorous brushing. Carbonate substrate was removed from the cell walls by dissolution in dilute acids (0.6 M Nitric acid) or Perenyi solution, after fixation in 3% formaldehyde solution in order to reduce damages to the enclosed endoliths. The decalcified algae were then squashed on a slide. The endoliths, free from the carbonate substrate, were observed using a light microscope.

Stereo light microscope: Specimens, broken perpendicularly to the surface were observed on a stereo light microscope, without any previous treatment.

RESULTS

Microbial boring in thalli of live coralline algae

In many rhodophyta and in particular in the crustose coralline algae, the filaments that compose their thallus, growing together, compose a sort of parenchyma. The upper external 20-30 rows of cells of each fil-

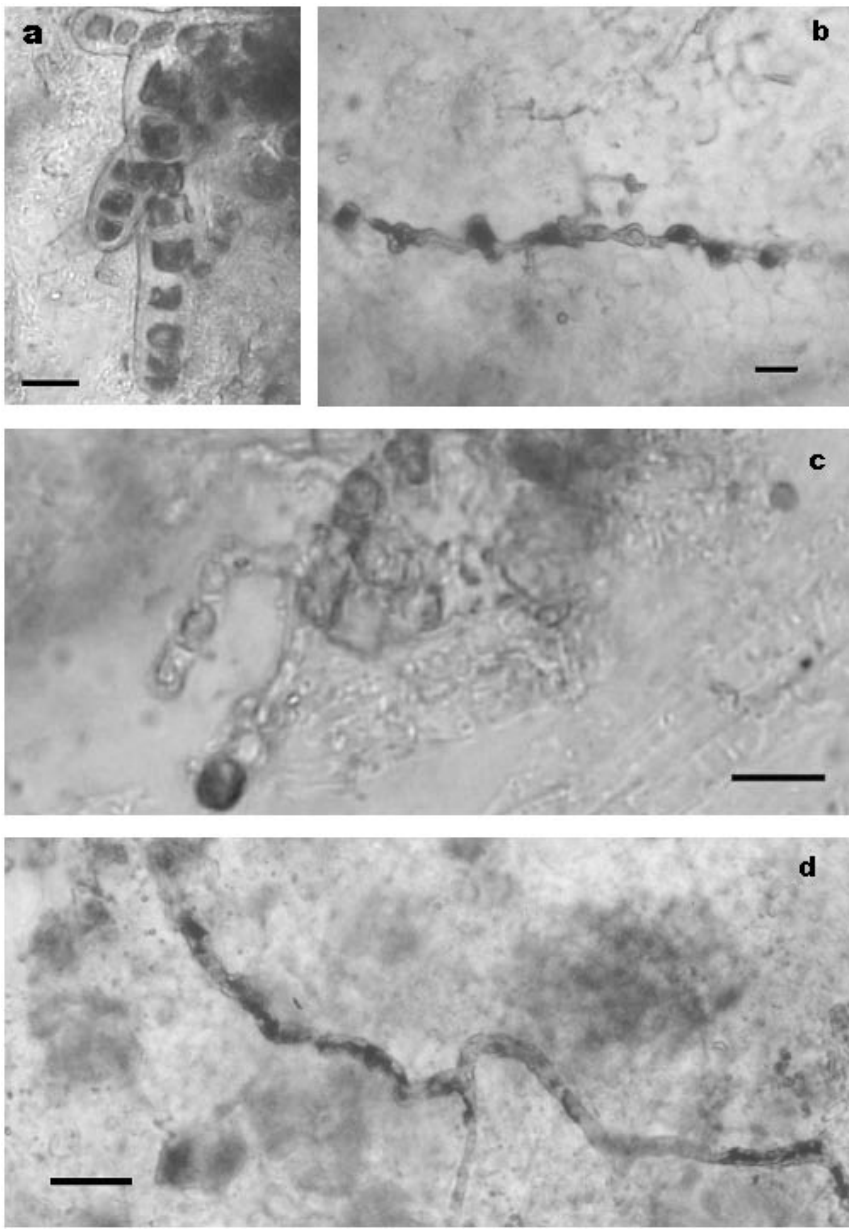

Figure 2. Microendoliths found in dead thalli of crustose coralline algae. (a) *Scopulonema hansgirgianum*. (b) *Phaeophila dendroides*. (c) *Mastigocoleus testarum*. (d) *Ostreobium quekettii*. Bar 10 μm.

ament form the live part of the alga, while the basal part dies.

It is very difficult to find endoliths in the live part of the coralline algae, as they are usually free from boring organisms. The microflora, if present in live thalli, consists almost exclusively of the cyanophyte *Plectonema terebrans* (Fig. 1a) accompanied some times by *Ostreobium quekettii* (Fig. 1c). *P. terebrans* was identified by means of its elongated shape, formed by cilindrical cells, measuring no more than 1-1.5 μm in diameter (Figs. 1 a,b). *Ostreobium quekettii* was observed only by means of light electron microscope. It is recognisable by means of its siphonous filaments with evident chloroplasts and the characteristic perpendicular branching. A picture of *O. quekettii*, found in a dead thallus, is visible also in Figure 2d.

Resin casts also show the presence of two different endoliths, distinguishable from one another for their dimensions. The thickest (2.5 μm) probably belongs to *Ostreobium quekettii*, whereas the thinnest (1-1.2 μm) is from *Plectonema terebrans* (Fig. 1 c).

Young thin red thalli of *Hydrolithon farinosum* and *Titanoderma pustulatum* and the superficial part of *Lithophyllum incrustans*, decalcified and observed by means of light electron microscope, highlighted these endoliths boring the cell walls. SEM observations were more precise to indicate the live thalli, where the organelles contained in the cells are visible. Nuclei and plastids fill the cells, whose walls are bored by endoliths (Figs. 1 a,b). In Figure 1b a cyanophyte sheet is visible inside the cell walls of live cells of *Lithophyllum incrustans*.

The boring action of endoliths follows the structure and the disposition of the host cell walls, being linear or like a flight of steps, according to the whim of the endolithic microorganisms. Therefore the penetration can be orthogonal or parallel to the surface of the thallus.

Numerous live thalli appeared completely free from endoliths.

Microbial boring in dead coralline algae thalli

Dead thalli are colonised by a microflora, which is more abundant than microflora boring the live thalli. The species composition of euendolithic microflora developing in dead thalli of *Lithophyllum* and *Titanoderma* was similar to that encountered in other carbonate substrates.

The reproductive structures of *Lithophyllum* (conceptacles) are colonised by cryptoendolithic microorganisms. The conceptacles, in fractured thalli observed using stereo light microscope, appear dark green coloured for the presence of vegetal organisms (Fig. 3).

In the central part of the fractured thallus a light green band is evident (Fig. 3). The pattern, which emerges, is that the coloured bands contain higher total density of green or brown filaments.

After decalcification, white dead thalli of *Lithophyllum incrustans* and T*itanoderma pustulatum* highlighted that they had been colonised by a microflora, which differs from the microflora colonising live coralline algae. It was composed most commonly by *Scopulonema hansgirgianum* ERCEGOVIC (Ercegovic, 1930) (Fig. 2 a), *Phaeophila dendroides* (CROUAN) BATTERS (Fig. 2 b), *Mastigocoleus testarum* LANGHERHEIM (Fig. 2 c), in addition to *Plectonema terebrans* and *Ostreobium quekettii* (Fig. 2 d).

Scopulonema hansgirgianum was found on the surface of the coralline algae as a thin brown layer of less than 1 mm thick. In the thalli, fractured perpendicularly on the surface, endolithic filaments can be seen penetrating inside and developing starting from epilithic filaments. The cells grow in all directions and have a pseudoparenchymatous aspect, where they appear compact and polygonal due to their reciprocal pressure. The cells have a diameter of 5–9 μm and are polygonally isodiametric or irregularly shaped. This characteristic of irregularity is typical of this algae. The lateral branching is the most frequent. At a first examination *S. hansgirgianum* could be confused with cyanobacterium *Hyella*, but the end cell of pseudofilaments of *Hyella* is longer and thinner. Moreover, both the presence of epilithic and endolithic filaments incline to believe for the S*copulonema genre*. According to Giaccone and Di Martino (1999) and Giaccone et al. (in press) *Scopulonema* and *Hyella belongs* to the same species *Entophysalis deusta* (MENEGHINI) DROUET and DAILY.

Mastigocoleus testarum is easily recognisable for the presence, at the apex of a filament, of heterocyst differently coloured compared to other cells. Heterocysts have a stratified sheath, visible at a great magnification or in sections observed by using transmission electron microscope.

Phaeophila dendroides is a septate siphonal chlophyte, whose cells have a 9–15 μm diameter. The parietal lobed chloroplasts contain numerous pyrenoids, as it is visible in Figure 2 b. It is the first endolithic microorganism found after the death of the calcareous algae.

Other endolithic microorganisms are observed in coralline algae, but at the moment they are not yet identified.

The above mentioned endoliths can penetrate the algal thallus from the surface, as it is visible in many specimens, where a crowd of traces are noticeable in the cell walls of the external rows of cells and decreases from the surface to the inner part of the thallus.

A lot of fungi colonize either the cell wall or the internal of the cells. Some images show fungi boring the cell wall, or crossing the cell transversally or longitudinally (Fig. 1 d).

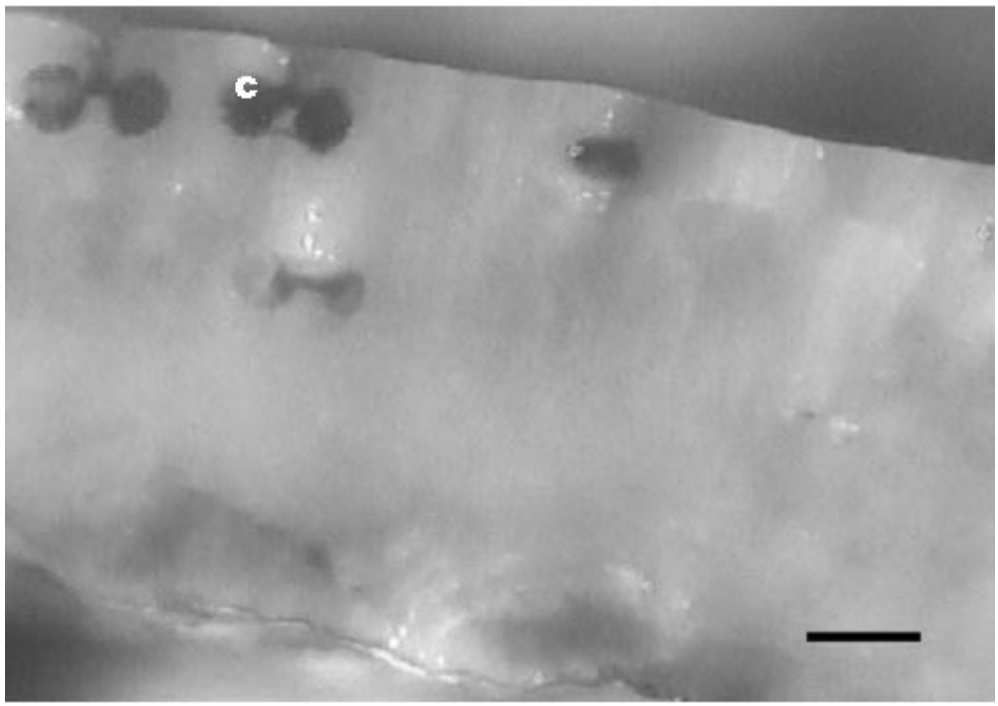

Figure 3. Green band, formed by a crossing of cyanobacteria and chlorophyta, visible in a dead thallus of *Lithophyllum*. Conceptacle (C). Bar 1 mm.

DISCUSSION

The difference between endolith assemblages inhabiting live and dead thalli of coralline algae can be explained considering the viability of the cells that constitute an alga. In fact dead thalli may be considered as any other calcareous substrate, such as shell fragments, carbonate sediments grains and coastal limestone, since only the calcified wall remains after the cell dies.

On the other hand cells of live calcareous algae are able to resist penetration of endoliths, by means of metabolic processes not yet cleared. Only the most tenacious among the endoliths succeed in overcoming this resistance in boring the walls of those cells still alive. The cyanobacterium which is more often found inside live thalli is *Plectonema terebrans* (Fig. 1 a,b), which was viewed either using light microscope or transmission and scanning microscopes (Ghirardelli, 1998).

In this case the influence of the light may be considered irrelevant, since the calcareous algae samples were all collected at the same depth and in very similar light conditions. In other cases (Ghirardelli, 1998) only *Plectonema terebrans* was found in algae collected at 15 metres depth in feeble light conditions, confirming therefore the characteristic of low light specialist of this cyanobacterium.

The chlorophyte *Ostreobium quekettii* was rarely extracted from still living thalli (Fig. 1 c).

In coralline algae the structure and the way of growth of the thallus allow the penetration to occur differently according to whether it is a live thallus or a dead thallus. The thallus structure of Corallinales is multiaxial and the crust expands though the division of the peripheral cells of the hypothallus (basal filament) and the division of the uppermost perithallus cells (Van den Hoek et al., 1995), so the only living part of the thallus is composed by the external 20-30 rows of cells. After some time, the basal oldest part of these filaments dies. Live cells oppose to boring to the majority of microborers, therefore allowing more easily and more frequently endoliths to enter from the lower part of the thallus, which is the one with a direct contact with the calcareous substrate on which the alga anchors. Boring ceases as soon as the endoliths reach the superficial living part of the thallus, which remains then free from any of the boring microorganisms. This could explain the presence of a green band (Fig. 3), formed by a crossing of a cyanobacteria and chlorophyta, which is visible in the cen-

tral part of the thicker thalli and which was also observed by Tribollet and Payri (2001) in *Hydrolithon onkodes*. It is, however, possible that the green band has been influenced by the best light conditions, since all the samples collected have grown in very good light conditions.

In dead thalli the entrance of endoliths occurs also from the surface part, or rather the outer layers are those more densely bored.

A limited presence of endoliths was also observed in live corals (Le Campion- Alsumard et al., 1995), whereas a more abundant microflora in a number of species and individuals was found in skeletons of dead corals meaning that "the specific composition of endoliths in live corals is the result of a selection of species, which can cope with the accretion rate of coral skeletons" (Le Campion- Alsumard et al., 1995).

The species composition of endoliths in live coral, colonized mainly by *Ostreobium quekettii*, is a little different from the composition of the microflora inhabiting the live coralline algae, in which *Plectonema terebrans* is the most frequent borer.

Moreover even among the endoliths found in calcareous algae and in skeletons of dead corals a slight difference may be observed: in Corallinales in the gulf of Trieste it is indeed possible to find together with *Mastigocoleus, Phaeophila, Ostreobium* and *Plectonema*, also *Scopulonema* [(*Entophysalis deusta*, according to Giaccone and Di Martino (1999) and Drovet (1981)] and other microendoliths not observed in corals and not yet identified. This is probably due to the different environment where the French Polynesian corals live and the Adriatic Sea where the coralline algae, mentioned in this paper, was collected. The endoliths living in the Northern Adriatic Sea are probably those described by Ercegovic in the Dalmatian coast (Ercegovic, 1930; 1932).

It is therefore unusual that organisms so different from one another, such as corals and coralline algae, behave so similarly towards endoliths: both in fact have developed defence mechanisms so as to reject, when alive, the attacks of endolithic microorganisms.

REFERENCES

Bak, R.P.M., Laane, W.P.M., 1987. Annual black bands in skeletons of reef corals (Scleractinia). Marine Ecology Prog. Series, 38, 169-175.

Chazotte, V., Le Campion-Alsumard, T., Peyrot-Clausade, M., 1995. Bioerosion rates on coral reef: interactions between macroborers, microborers and grazers (Moorea, French Polynesia). Palaeogeography, Palaeoclimatology, Palaeoecology, 113, 189-198.

Drovet, F., 1981. Summary of the classification of Blue-green Algae. Cramer ed, Vaduz, ed. 135-209.

Ercegovic, A., 1930. Sur quelques types peu connus des Cyanophycées lithophytes. Archiv Protistenk, 71, 361-373.

Ercegovic, A., 1932. Etudes écologiques et sociologiques des Cyanophycées lithophytes de la cote yugoslave de l'Adriatique. Bull. Int. Acad. Sci. Arts Cl. Sci. Math. Nat., 26, 33-56.

Ghirardelli, L.A., 1998. An endolithic cyanophyte in the cell wall of calcareous algae. Botanica Marina, 41, 367-373.

Giaccone, G., Di Martino, V., 1999. Biologia delle alghe e conservazione dei monumenti. Bollettino Accademia Gioenia Scienze Naturali, 32, 53-81.

Golubic, S., Brent, G., Le Campion, T., 1970. Scanning electron microscopy of endolithic algae and fungi using a multipurpose casting-embedding technique. Lethaia, 3, 157-161.

Golubic, S., Friedmann, I., Schneider, J., 1981. The lithobiontic ecological niche, with special reference to microorganisms. Journal of Sedimentary Petrology, 51, 475-478.

Kendrick, B., Risk, M.J., Michaelides, J., Bergman, K., 1982. Amphibious microborers: Bioeroding fungi isolated from live corals. Bulletin of Marine Sciences, 32, 862-867.

Laborel, J., Le Campion-Alsumard, T., 1979. Infestation massive du squelette de coraux vivants par des Rhodophycées de type Conchocelis. Comptes rendus de l'Academie des Sciences Paris, 288, 1575-1577.

Le Campion-Alsumard, T., Golubic, S., Hutchings, P., 1995. Microbial endoliths in skeletons of live and dead corals: *Porites lobata* (Moorea, French Polynesia). Marine Ecology Progr. Series, 117, 149-157.

Littler, M.M., Littler, D.S., 1995. Impact of CLOD pathogen on Pacific coral reefs. Science, 237, 1356-1360.

Lukas, K.J., 1974. Two species of the chlorophyte genus *Ostreobium* from skeletons of Atlantic and Caribbean corals. Journal of Phycology, 10, 331-335.

Nichetto, P., Ghirardelli, L.A., 1994. A method to access areal vitality at first sight. Bollettino della Società Adriatica di Scienze, 75, 269-275.

Peyrot-Clausade, M., Hutchings, P.A., Richard, G., 1992. The distribution and successional patterns of macroborers in marine *Porites* at different stages of degradation on the barrier reef, Tiahura, Moorea, French Polynesia. Coral Reefs, 11, 161-166.

Reynolds, E., 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. Journal of Cellular Biology, 17, 208-217.

Spurr, A.R., 1969. A low viscosity epoxy resin embedding medium for electron microscopy. Journal of Ultrastructural Research, 26, 31-42.

Tribollet, A., Payri, C., 2001. Bioerosion of the coralline alga *Hydrolithon onkodes* by microborers in the coral reefs of Moorea, French Polynesia. Oceanologica Acta, 24, 329-342.

Van Den Hoek, C., Mann, D.G., Jahns, H.M., 1995. Algae: an introduction to phycology. Cambridge University Press, 627 pp.

Weber-Van Bosse, A., 1932. Algues. Résultats Scientifiques du Voyage aux Indes Orientales Néerlandaises de LL. AA. RR. le Prince et la Princesse Leopold de Belgique. Museum royale d'Histoire Naturelle (Belgique) Mémoires Hors Série, 6, 1-28.