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Gill Ultrastructure and Symbiotic Bacteria in the Tropical Lucinid, *Linga pensylvanica* (Linné)

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Abstract

The cellular organization of the gill filament is described in the lucinid, *Linga pensylvanica*, whose gill cells host the same species of sulfur-oxidizing bacterial endosymbionts as *Codakia orbicularis*, another inhabitant of similar tropical sea-grass beds. In *L. pensylvanica*, the ciliated zone is shorter and the intermediary zone is more elongated than they are in the gill filament of Lucinidae described to date. Secretory cells appear to be much diversified, with three secretory cell types; one classical mucous cell type interspersed with bacteriocytes and intercalary cells in the lateral zone, and two secretory cell types located at the limit of the intermediary zone and lateral zone. Bacteriocytes have been observed to have direct contact with pallial sea water even so intercalary cells overlap partly their apical microvilli. Peroxisomes which have not been identified in the gill cells of other symbiotic bivalves are common organelles in the bacteriocytes of *L. pensylvanica*. To compare gill structures in *L. pensylvanica*, *C. orbicularis*, and *Lucina pectinata* shows that each species has unique features. Significant differences between *C. orbicularis* and *L. pensylvanica* gill structure have not received a straightforward interpretation so far.

Keywords: Symbiosis, bacteria, tropical Lucinidae, ultrastructure, peroxisomes

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1. Introduction

Since their discovery by Felbeck et al. (1981) in deep-sea hydrothermal-vent fauna, symbioses between bivalves and sulfur-oxidizing chemoautotrophic bacterial endosymbionts located in gill cells have been identified in various environments. As emphasized by Reid (1990), the sulphide-oxidizing symbiosis has been found in all species of the eight genera of Lucinidae that have been examined and such a symbiosis appears to be a major evolutionary trend in this family. In the shallow-water environment of tropical sea-grass beds, the dominant species of bivalves belong to the family Lucinidae (Jackson, 1973), among which some Caribbean species have already been examined by Giere (1985). However, neither of the three large species, identified according to Abbott (1974), *Codakia orbicularis* (Linné, 1758), *Linga pensylvanica* (Linné, 1758), and *Lucina pectinata* (Gmelin, 1791) have been included in this investigation. Symbiotic bacteria were first identified in the gills of *C. orbicularis* by Berg and Alatalo (1984) and the gill structure of this species was described by Frenkiel and Mouëza (1995). Moreover, phylogenetic studies using comparative analysis of 16S rDNA sequences of these two tropical Lucinidae endosymbionts showed that, though belonging to different genera, *C. orbicularis* and *L. pensylvanica* which live in the same type of shallow-water sea-grass beds seem to host identical intracellular gill symbionts (Durand et al., 1996). Conversely, *L. pectinata* which lives in black reducing mud of mangrove swamps hosts a symbiont which is considered as the most divergent microorganism from the cluster of Lucinacea symbionts (Durand et al., 1996). This species appears as much different from *C. orbicularis* with regards to its hemoglobin supply (Read, 1962; Kraus and Wittenberg, 1990) and to its gill structure (Frenkiel et al., 1996).

The aim of this paper is to describe the cellular organization of the gills of *L. pensylvanica* and to compare it with that of other tropical Lucinidae already described and especially with the gill structure of *C. orbicularis*, which lives in a similar habitat, and *L. pectinata* which lives in a very different one.

2. Materials and Methods

Specimen collection

Adult specimens of *Linga pensylvanica* (Linné, 1758), collected by hand on the edge of shallow water sea-grass beds in Martinique, were kept alive in filtered sea water until fixation which was performed within one week.

Histology and histochemistry

An overall view and some histochemical information were obtained from paraffin sections. Gills were fixed in Bouin's fluid prepared in sea water and embedded in paraplast after dehydration. Sections (7 µm thick) were stained by various histological techniques performed according to Gabe (1968). We used Goldner's trichrome for morphological observation, Alcian blue staining (pH 1 or 3) to discriminate the type of mucosubstances, and periodic acid Schiff (PAS) reaction to reveal glycoconjugates. A combined staining with Alcian blue and Alcian yellow allows discrimination between glycosaminoglycans (stained blue) and sialomucins (stained yellow). The general identification of proteins by Danielli's tetrazoreaction was performed on gills fixed in 4% formaldehyde.

Scanning Electron Microscopy (SEM) preparation

Both intact and delaminated gill tissues were fixed in 2.5% glutaraldehyde in sea water for 24 hours at room temperature. After a rinse in 0.1 M pH 7.2 cacodylate buffer adjusted to 1,000 mOsM by NaCl, gill pieces were dehydrated in graded acetone series and critical point dried using CO_2 as transitional fluid. Then, they were sputter coated with gold before observation in a SEM Hitachi S-2500 at 20 kV.

Transmission Electron Microscopy (TEM) preparation

Small pieces of gills were fixed for two hours at 4°C in 2.5% glutaraldehyde in 0.1 M pH 7.2 cacodylate buffer adjusted to 1,000 mOsM with NaCl or with a saline solution designed for marine invertebrates (Hernandez-Nicaise and Amsellem, 1980). After a brief rinse, they were postfixed for one hour at 4°C in 1% osmium tetroxide in the same buffer, then rinsed in distilled water and treated by aqueous 2% uranyl acetate for one more hour. Pieces were then rinsed in distilled water, dehydrated through a graded ethanol series, and embedded in Epon-Araldite according to Mollenhauer (in Glauert, 1975), or in the acrylic resin LR White. Sections were cut using an Ultracut E Reichert ultramicrotome; semi-thin sections (0.5 µm thick) were stained with 0.5% toluidine blue in 1% borax and thin sections (60 nm thick) were contrasted with aqueous uranyl acetate and lead citrate before being examined in a TEM Hitachi H-8000 at 100 kV.

3. Results

General description of the gill

The light-brown single demibranchs of *L. pensylvanica* are homorhabdic and a faint groove is present along their ventral margin (Fig. 1). Observed in paraffin sections, the descending and ascending lamellae linked by lacunar tissular bridges delimit an "empty" interlamellar space, which means that it contains clear sea water, but no stainable secretory product. Each filament is composed of 3 distinct regions: a short ciliated zone, a relatively large intermediary zone, and a lateral zone, some three-fold larger than the two others (Fig. 2). Each gill filament consists of a simple epithelium, which is in contact with a connective axis at the level of the ciliated zone, and encloses a large blood lacuna in the lateral zone.

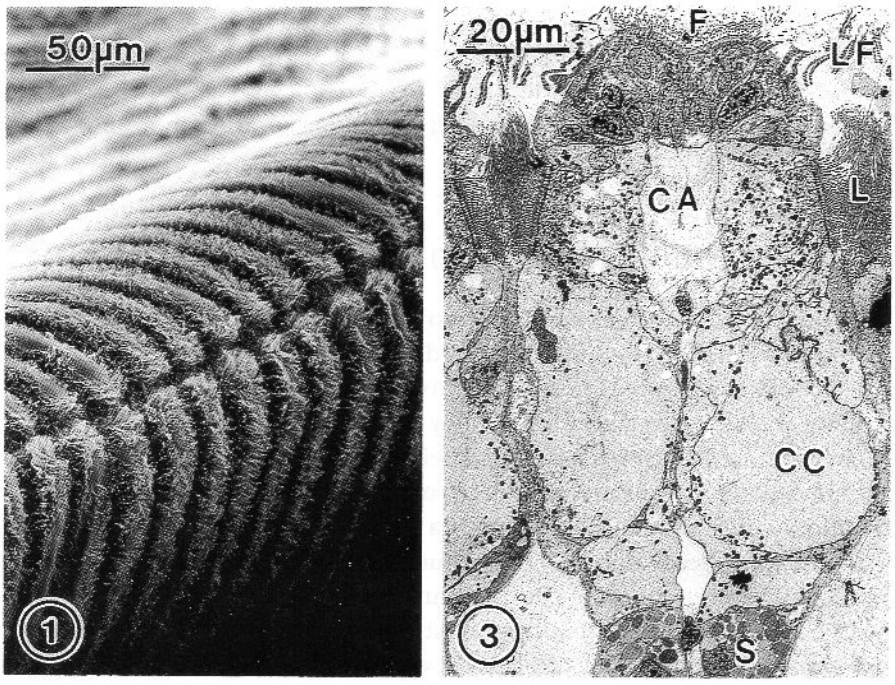

Figure 1. SEM view of the faint groove along the ventral margin of the homorhabdic gill.
Figure 3. TEM of the ciliated and intermediary zones. Frontal (F), laterofrontal (LF), and lateral (L) ciliated cells are the main cell types of the ciliated zone organized along a collagen axis (CA). Large clear cells (CC) make up the main part of the intermediary zone; they are in contact with secretory cell types (S).

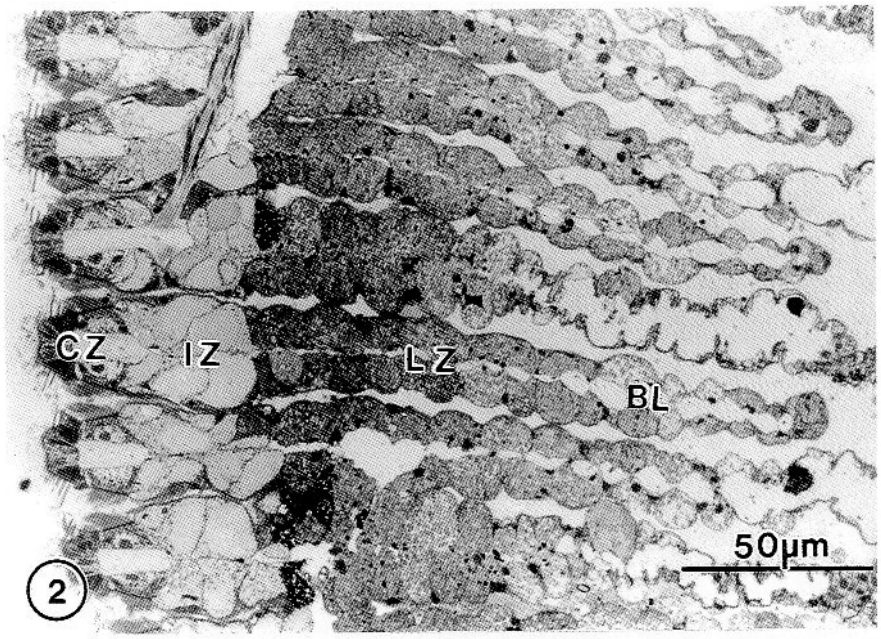

Figure 2. Light micrograph of a semi-thin transverse section of the outer lamina. Each gill filament consists of a simple epithelium in which the lateral zone (LZ) is in contact with a blood lacuna (BL) and is separated from the short ciliated zone (CZ) by a large intermediary zone (IZ).

Gill filament structure

The short ciliated zone (20 to 25 µm) displays a typical ciliation which consists of short frontal cilia, fused laterofrontal cilia featuring 2 rows of long cirri, and long lateral cilia (Fig. 3). The laterofrontal and lateral cilia are intermingled between adjacent filaments, and thus form an efficient filtering device. The eulateral cells appear to make up a cluster composed of three different cell types (Fig. 4). Two central cells possess cilia all along their apical surface. These cells are bordered on each side by cells which display cilia only on a limited part of their apical surface which is adjacent to the main ciliated cells. The first one, near the prolateral cell, is rather inconspicuous, whereas the last one is a very large clear cell with some 10 rows of cilia and numerous mitochondria.

The intermediary zone which is longer than the ciliated zone, 30 to 35 µm and 20 to 25 µm, respectively (Fig. 2), is made up of several cell types. Up to

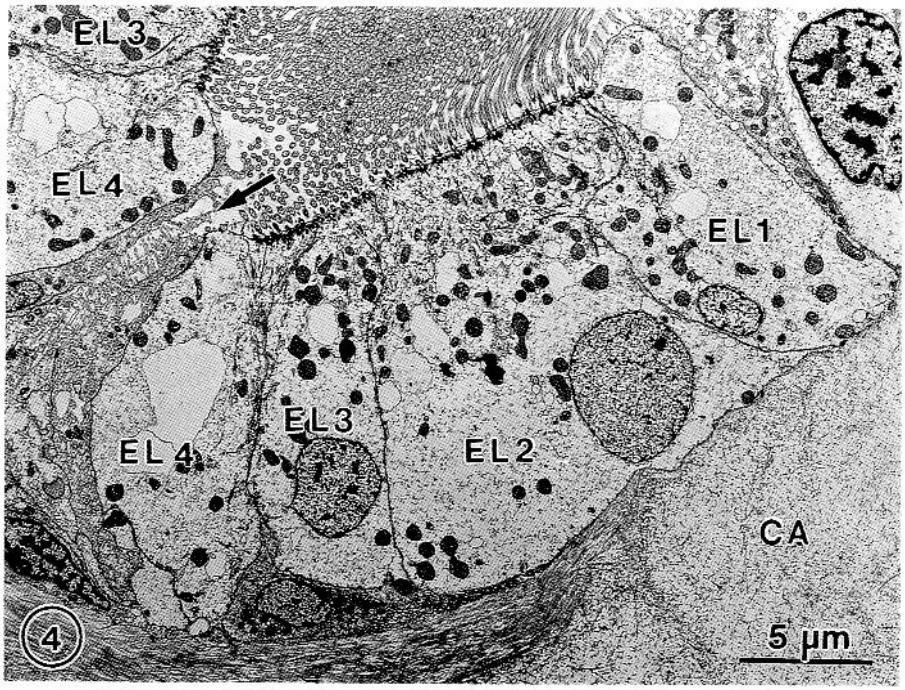

Figure 4. TEM of the ciliated zone organized along the collagen axis (CA). The eulateral cells (EL1-EL2-EL3-EL4) comprise three cell types. EL2 and EL3 possess cilia all along their apical surface, whereas EL1 and EL4 display cilia only on the limited part of their apical surface respectively adjacent to EL2 and EL3. Arrow indicates the orientation of the sea water flow towards the bacteriocytes.

four electron-lucent large cuboidal cells with numerous mitochondria distributed mostly along the cell membrane are its main components (Fig. 3). The first one which is partly ciliated appears to be simultaneously part of the ciliated cell-group and of the intermediary zone. Although they are very similar in aspect, the other ones are non-ciliated. Together with those of the adjacent filament, these cells constitute a narrow canal between the ciliated zone and the lateral zone. Some elongated cells with comparatively large nuclei are stretched to overlay them as well as the muscular and connective tissues which link adjacent filaments together, making a sheath along the wall of the canal (Fig. 5).

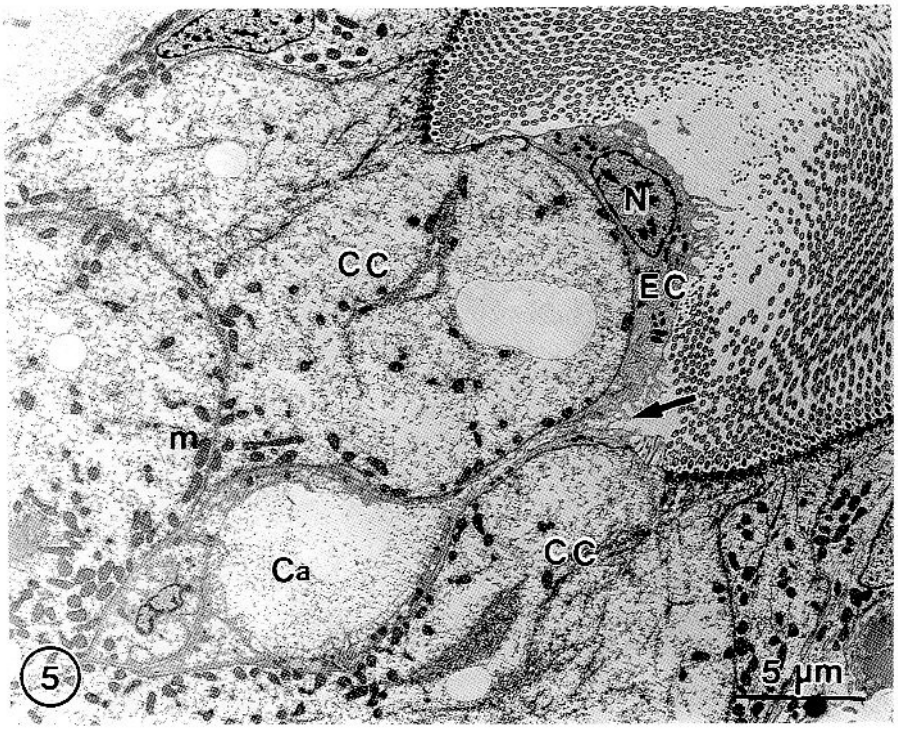

Figure 5. TEM of the intermediary zone of adjacent filaments. Elongated cells (EC) with apical nuclei (N) overlay the large clear cells (CC) which contain numerous mitochondria (m) and delineate the wall of the canal (Ca) where secretory cells excrete mucus which forms a film along the ciliated-part of the filament. The large clear cells constitute the narrow aperture (arrow) which directs sea water to the bacteriocyte channel.

Moreover, two secretory cell types are encased between the intermediary cells and the most superficial bacteriocytes of the lateral zone; in addition, a few of them are located between the ciliated cells and intermediary cells. These secretory cells have been characterized by histochemical techniques and by their ultrastructural features. In either secretory cell type, the nucleus, ranging from 6 to 7 µm in diameter, is at the basis of the cell near the blood lacuna. The first secretory cell type, 20 to 25 µm long and 10 to 15 µm wide (Fig. 6), is characterized by homogeneous osmiophilic granules (2 to 4 µm in diameter) which are stained blue by toluidine blue on semi-thin sections. These granules are PAS reactive and are detected by the tetrazoreaction. Therefore, their main secretory product is a glycoprotein; their faint reaction to Alcian

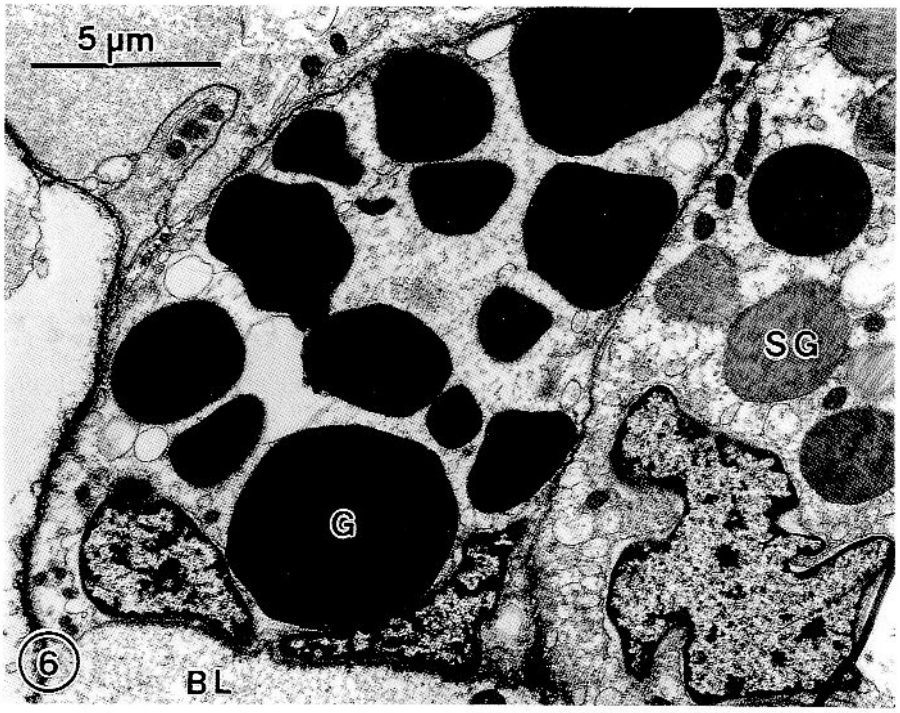

Figure 6. TEM of two adjacent secretory cell types. The first one is characterized by homogeneous osmiophilic granules (G), the second one by numerous small granules (SG) occupied by a tubular network. The nucleus of each secretory cell is located in its basal part near the blood lacunar space (BL).

yellow appears to be indicative of a non-sulfated sialomucine. The remaining cytoplasmic area contains some organelles such as mitochondria but no conspicuous Golgi apparatus. Near the apical pole of the cell, the osmiophilic granules become larger and lighter. The second secretory cell type (Fig. 6) contains numerous smaller granules not stained by toluidine blue which fill up the cytoplasm of the cells. Unlike the first secretory cell type, numerous Golgi stacks and mitochondria are frequently observed in the cytoplasm of this second secretory cell type (Fig. 7) which looks like mucocytes. However, its granules are not characterized by the histochemical techniques used to identify mucosubstances and proteins. They are occupied by a regular network of microtubular structures (Figs. 7 and 8); their size and density seem to depend on their maturation.

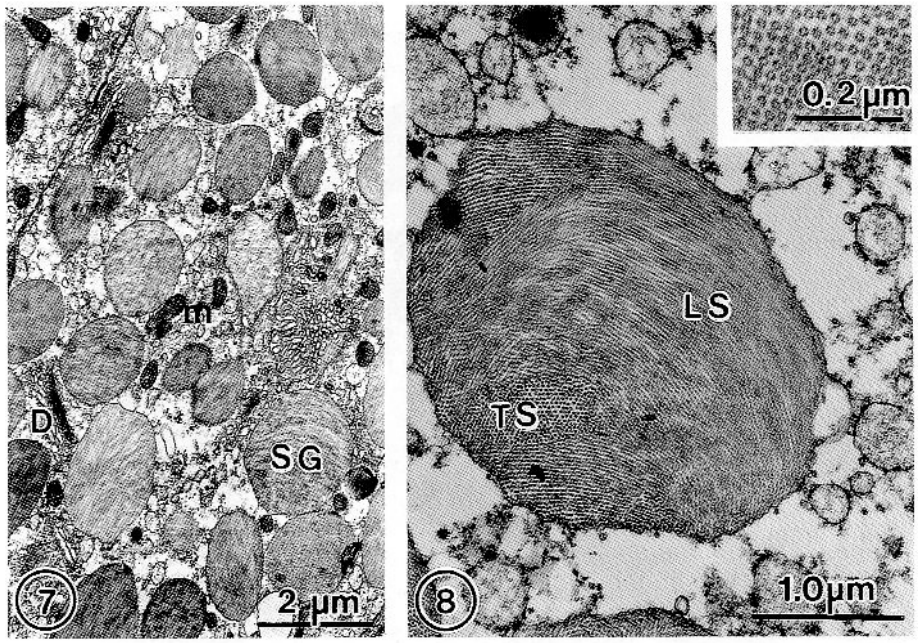

Figure 7. TEM view of the cytoplasmic volume of the second secretory cell type in which numerous Golgi dictyosomes (D) and mitochondria (m) are present. The size and the density of secretory granules (SG) depend on their maturation.

Figure 8. TEM. A higher magnification of a small granule of the second secretory cell type present in the intermediary zone of *L. pensylvanica* gill filament shows transverse section (TS) and longitudinal section (LS). The aspect of the network is different according to various orientations. Transverse section of the tubular network found in the granules of the second secretory cell type.

The lateral zone constitutes the main part of the filament and accounts for the thickness of the gill (Fig. 2); its innermost part is occupied by a large hemocoele. Bacteriocytes, from 25 to 35 µm long and about 15 µm wide (Fig. 9), are the most prevalent cell type to be observed in this zone. The bacteriocyte nuclei are usually displaced to the base of the cell near the blood lacuna. The rounded apical surface differentiates regular microvilli linked by a fibrous glycocalyx (Figs. 9 and 18), whereas numerous folds of the basal membrane occur in contact with the blood lacuna of the filament axis (Fig. 9). The most conspicuous structures in the bacteriocyte are the bacteria. Even so, its cytoplasmic volume contains various organelles, mostly mitochondria, lysosomal residual bodies at various degrees of maturity (Fig. 12), and smooth endoplasmic reticulum profiles associated to the lateral and basal cell-

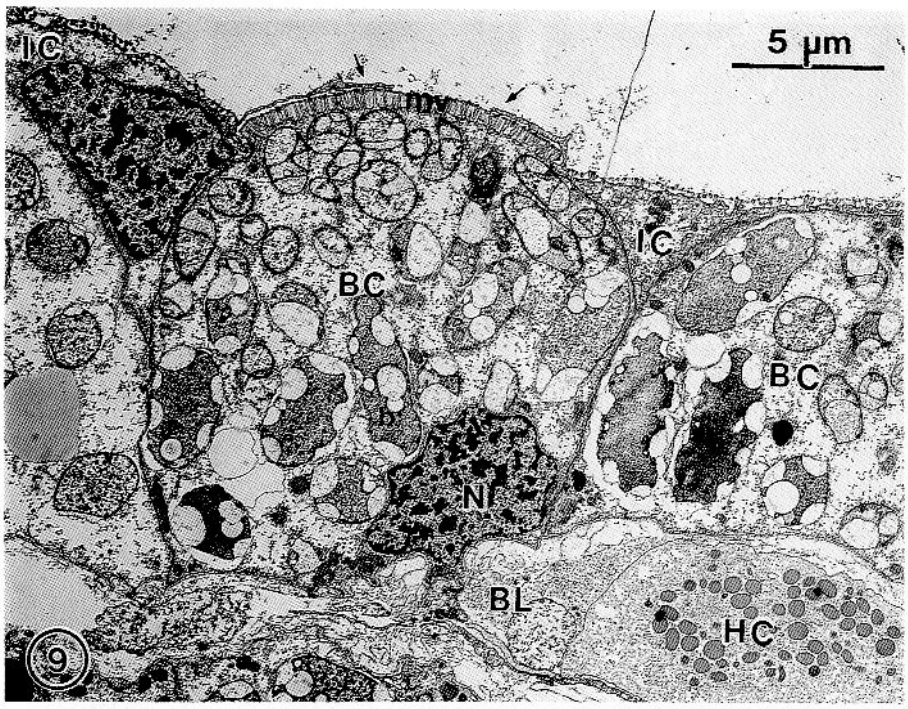

Figure 9. TEM. Intercalary cells (IC) are interspersed among bacteriocytes (BC). Bacteriocytes possess a basal nucleus (N) near the blood lacuna (BL) of the filament-axis and endosymbionts (b) occupy the main part of the cytoplasmic volume. Marginal expansions of intercalary cells (arrows) expand over the apical microvilli (mv) of the bacteriocyte thus limiting its contact with pallial sea water. A hemocyte (HC) is contiguous with the basal membrane inside the blood lacuna.

membrane (Fig. 14). Moreover, in each bacteriocyte, all along the gill filament, some membrane-bound inclusions (0.5 µm in diameter) which are generally located near the bacterial vacuoles (Figs. 15 and 16) have been identified as peroxisomes with regard to their typical paracristalline core (Fig. 15). These peroxisomes are generally surrounded by smooth endoplasmic reticulum profiles.

Intercalary cells with a narrow basis and an enlarged apical area covered by microvilli are regularly interspersed with bacteriocytes (Figs. 9 and 10). They have a rounded or elongated nucleus in an apical position, and some mitochondria which apparently are their sole organelles. TEM and SEM observations show that these intercalary cells encroach upon the apical area of the bacteriocytes and restrict their contact with pallial sea water (Figs. 9–11).

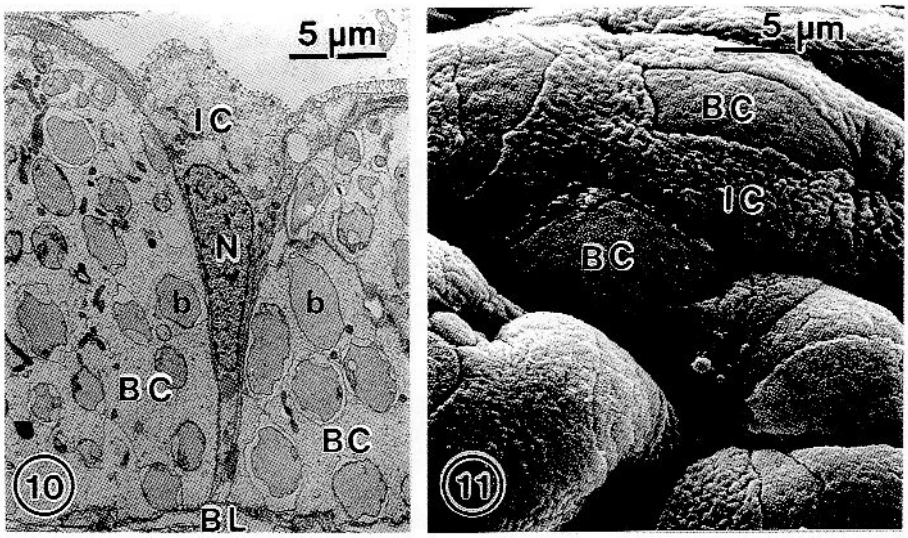

Figure 10. TEM. Intercalary cells (IC) have a narrow basis with an elongated nucleus (N) located in an apical position; bacteriocytes (BC); bacterial endosymbionts (b); blood lacuna (BL).

Figure 11. SEM. The bacteriocytes (BC) and intercalary cells (IC) make up an intricate array of small and large apical surfaces respectively, all covered with microvilli.

A third secretory cell type, intermingled with bacteriocytes and intercalary cells, elaborates typical proteoglycans detected by a red metachromasia with toluidine blue, by their reactivity to Alcian blue at pH 1 and pH 3, and by their negative response to PAS reaction. Thus, they are mucocytes with typical corresponding ultrastructural features (data not shown).

Numerous axons and muscle fibers are found all along the blood lacuna from the intermediary zone to the abfrontal end of each gill filament. A nerve with multiple axons is always present at the end of each filament facing the interlamellar space (Fig. 17). However, no direct relationship with the basal part of intercalary cells or bacteriocytes has been identified.

Fine structure of bacterial symbionts

Bacteria are individually enclosed or, less frequently, dividing within vacuoles (Figs. 9, 10, and 18). These endosymbionts are rod-shaped with the characteristic double-membrane of gram-negative bacteria (Fig. 18). They are

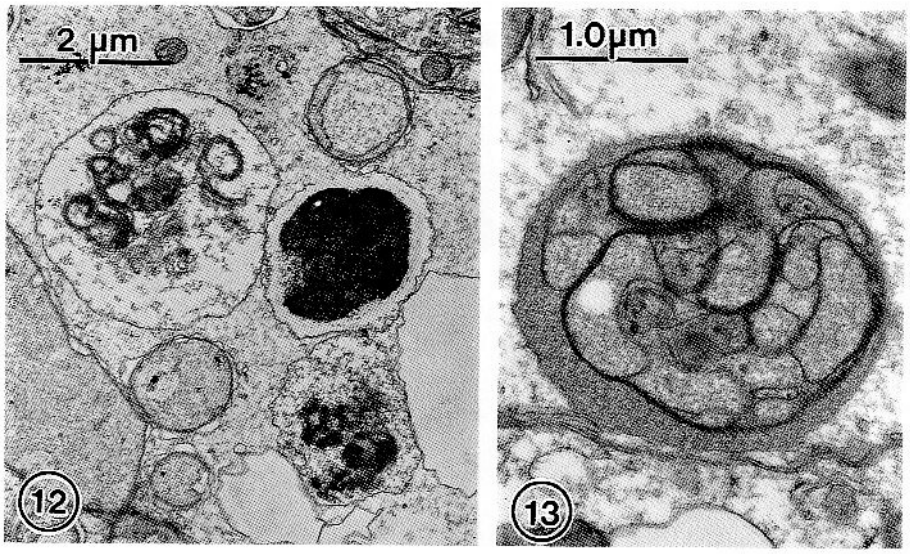

Figure 12. TEM view of three lysosomes at various degrees of maturity in one and the same bacteriocyte.
Figure 13. Complex lysosomal structure showing partly destroyed-bacteria.

large (0.8 to 2.4 µm × 1.5 to 5 µm) and some ovoid-shaped figures are probably due to the section orientation. Partly destroyed bacteria are not infrequent and may be, on some occasions, included in complex lysosome-like structures (Fig. 13). The bacterial cytoplasm contains membrane-bound, apparently empty vesicles of variable sizes which are, in fact, located in the periplasmic space (Figs. 9, 10, 17, and 18). Moreover, the bacterial cytoplasm contains numerous non-membrane-bound irregular inclusions (25 to 50 nm in diameter) slightly polygonal in shape and considered to be storage granules (Fig. 18). The nuclear area quite frequently encompasses an annular structure composed of a variable number of clear and dark laminae, about 1.7 nm thick (Figs. 18 and 19), never associated with the cell membrane.

4. Discussion

The presence of endocellular chemoautotrophic bacteria has already been demonstrated by enzymological and electron microscopic studies in several

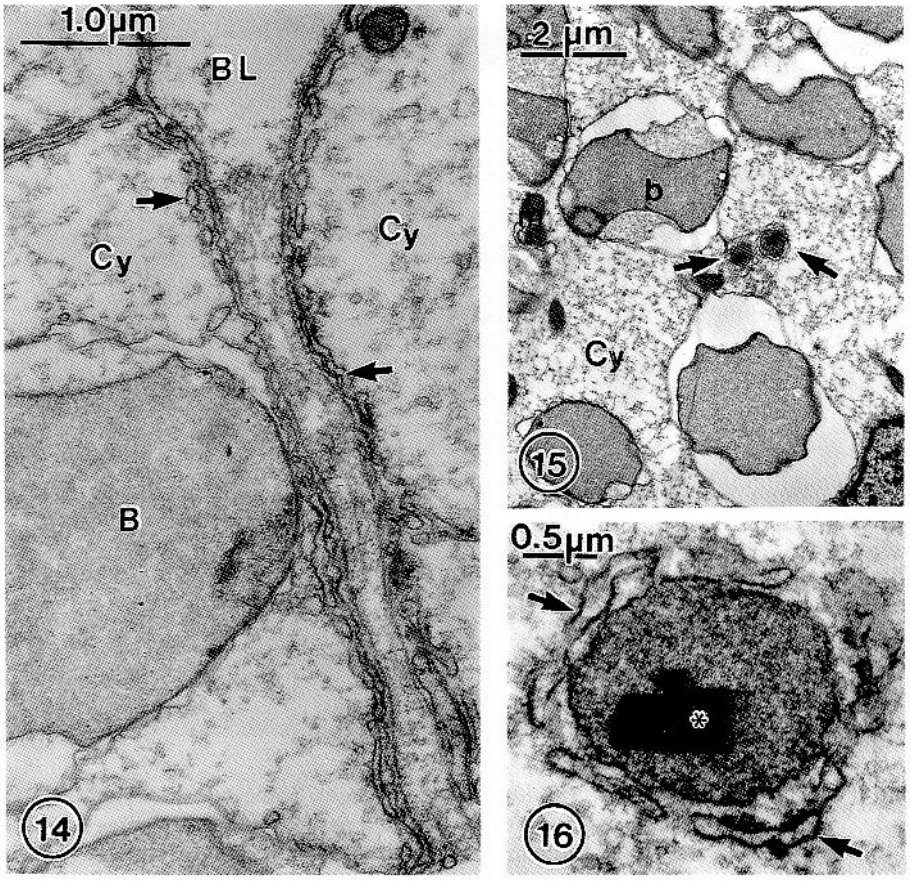

Figure 14. Smooth reticulum (arrows) is frequent along the lateral and basal cell-membranes of bacteriocytes; bacteria (B); blood lacuna (BL); bacteriocyte-cytoplasm (Cy).

Figure 15. TEM view of two peroxisomes (arrows) found in the bacteriocyte-cytoplasm. They do not seem to occupy a specific area in the cytoplasm-volume (Cy), but they are often located near the envacuoled chemoautotrophic bacteria (b); mitochondria (m).

Figure 16. TEM of a peroxisome surrounded by smooth reticulum endoplasmic (arrows) at a higher magnification. Note the paracristalline electron-dense inclusion (asterisk).

Lucinacea (for a review, see Reid, 1990). The tropical lucinid *Linga pensylvanica* occupies similar shallow-water sea-grass beds as the previously studied *Codakia orbicularis* and they appear to host the same species of

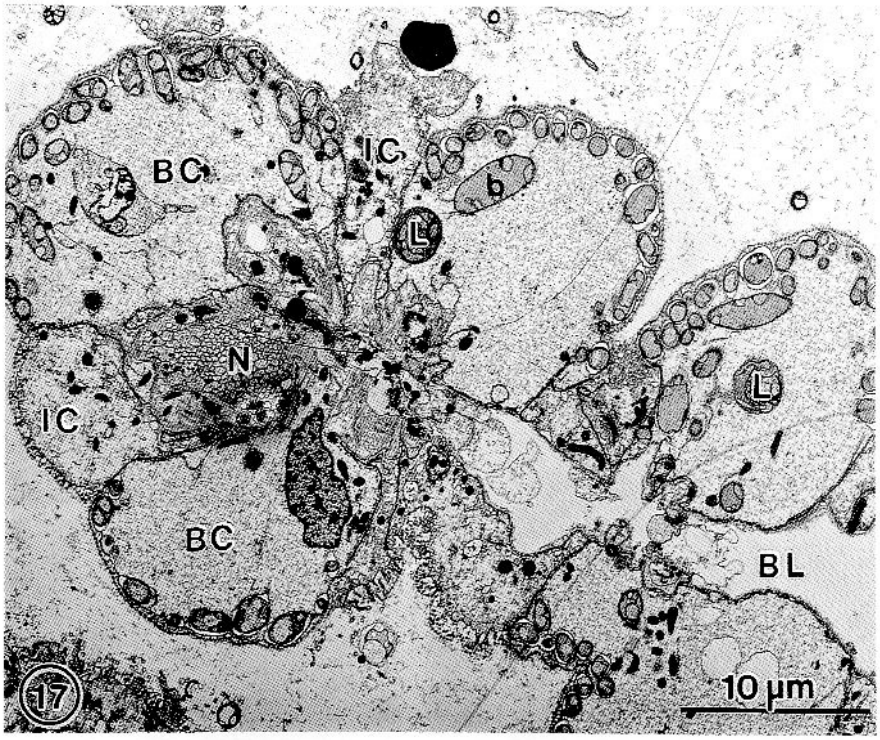

Figure 17. Abfrontal end of *L. pensylvanica* gill filament. Some bacteriocytes (BC) and intercalary cells (IC) are contiguous with a nerve (N). Bacteriocytes bordering the blood lacuna (BL) contain some lysosomes (L) and endosymbiotic bacteria (b) which are located in the superficial area of the bacteriocyte-cytoplasm.

eubacterial endosymbiont (Durand et al., 1996). However, in *L. pensylvanica,* the gill filament structure and the bacterial association appear to be different from those observed in *C. orbicularis* (Frenkiel and Mouëza, 1995) and equally different from those observed in *Lucina pectinata* (Frenkiel et al., 1996) which inhabits the muddy bottom of mangrove swamps.

Structural relationship between cells

The ciliated zone of the gill filament of *L. pensylvanica* comprises the same cell types as those of previously described Lucinidae; however, its frontal and laterofrontal parts appear to be flattened and the ratio between the ciliated zone and intermediary zone is unlike that described in *C. orbicularis,* as the

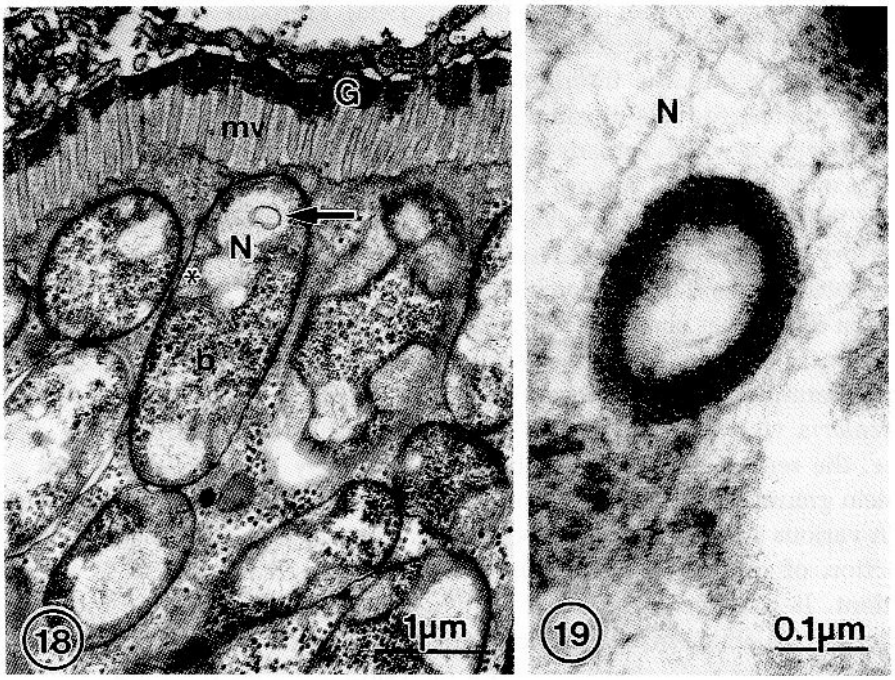

Figure 18. TEM of endocellular bacteria (b) tightly enclosed inside bacteriocyte-vacuoles. An annular structure (arrow) is present in the DNA area (N) of a bacterium. The bacterial cytoplasm contains numerous non-membrane-bound vesicles (probably glycogenic storage), and membrane-bound vesicles located in the periplasmic space (probably sulfur storage, asterisk). Bacteriocyte-microvilli (mv) are linked at their apical part by a dense glycocalyx (G) and are covered by slender expansions of adjacent intercalary cells (ice).

Figure 19. Higher magnification of the lamellar structure located in the nuclear area (N) of bacteria. This annular structure is composed of a variable number of alternatively dark and clear laminae, about 1.7 nm thick.

ciliated zone is much shorter and the intermediary zone much more developed in *L. pensylvanica*. The intermediary zone is composed of a few large clear cells – two to four cells – which are located between the ciliated lateral cell group and the lateral zone. These cells are almost devoid of organelles with the exception of numerous mitochondria mostly distributed along the cell membrane. Similar intermediary cells have been considered as storage-cells by Reid and Brand (1986) in view of the presence of glycogen granules. In *L. pensylvanica*, these cells appear to form the narrow aperture of large bacteriocyte channels similar to those described in other Lucinidae (Distel and

Felbeck, 1987; Frenkiel and Mouëza, 1995; Frenkiel et al., 1996). This arrangement allows a regulated water-flow along the bacteriocytes from the mantle cavity into the interlamellar space. Both secretory cell types may excrete their products through this intermediary-zone canal either as a mucosubstance which spreads upon the ciliated zone or as a secretory product which may be carried toward the interlamellar space by the sea water-flow along the lateral zone. However, the secretory cell type 2 contains peculiar secretory granules which have not been identified either as glycoproteins or as mucosubstances and its function is still unknown.

Most differences observed between species occur in the lateral zone of the gill filament. In *L. pensylvanica*, bacteriocytes are to be found all along the lateral zone intermingled with intercalary cells, as compared to the bacteriocytes in *C. orbicularis* which occupy only the most superficial one-third of the lateral zone, the remaining two-thirds being occupied by large cells crowded with protein granules (Frenkiel and Mouëza, 1995).

In various species, the bacteriocytes are specialized to house bacteria but the function of other cell types which have few typical features is far from evident. It has been suggested that intercalary cells could be either new bacteriocytes (Fiala-Médioni and Métivier, 1986) or storage-cells (Reid and Brand, 1986) or cells able to dispose of residual bodies (Distel and Felbeck, 1987). In *Lucina floridana*, it was implied that intercalary cells were isolating the bacteriocytes from sea water (Fisher and Hand, 1984), whereas Giere (1985) considered that this result was due to an oblique section plane. Distel and Felbeck (1987) also considered that intercalary cells were forming a thin layer between bacteriocytes and the lumen of the bacteriocyte channel. The direct observation of the apical surface of the gill filaments with SEM allows us to support Giere's conclusion and to consider that Distel and Felbeck's interpretation was also questionable. In the three species analysed, we noticed that the relations between bacteriocytes and intercalary cells are variable, but that the bacteriocytes keep a direct contact with sea water (Frenkiel et al., 1995; 1996). In *L. pensylvanica*, the bacteriocytes differentiate microvilli on a restricted apical area limited by large intercalary cells which encroach upon them. These intercalary cells develop a circular flange which overlaps the apical microvilli of the bacteriocytes to a variable extent also in *L. pectinata*. They should be able to control the extent of contact of the bacteriocytes with pallial sea water. However, in *L. pensylvanica*, the intercalary cells lack the long slender expansions supposed to be chemosensory in *L. pectinata* (Frenkiel et al., 1996). Conversely, in *C. orbicularis*, (Frenkiel and Mouëza, 1995) the intercalary cells are narrow and the bacteriocytes are in contact with sea water through a large free apical area. The relationships between intercalary cells and bacteriocytes, and the apical differentiations of the intercalary cells

appear to be much variable according to species. However, the possible adaptive and evolutionary significance of intercalary cells are still unclear.

The bacteriocytes of *L. pensylvanica* contain numerous bacteria but the bacterial vacuoles, which are not as crowded as in *C. orbicularis* bacteriocytes, allow enough cytoplasmic space for organelles such as mitochondria, lysosomal residual bodies and peroxisomes to be conspicuous. The content of the secondary lysosomes, in the bacteriocytes of *L. pensylvanica*, suggests a possible lysosomal resorption of the eubacterial endosymbionts which would be a way for organic material to be transferred from the bacteria to the host as supposed by Fiala-Médioni et al. (1986) and by Herry et al. (1989). This process may serve as a source of fixed carbon for the host or as a means of preventing excessive proliferation of bacterial endosymbionts in each bacteriocyte (Le Pennec et al., 1988). This digestion of the symbionts has to be part of the turnover of the endosymbionts which involves the cellular division of the symbionts themselves, or a continuous infestation of the bacteriocytes by free-living symbiont forms. A similar type of lysosomal activity was described in *C. orbicularis* by Frenkiel and Mouëza (1995) as in other symbiotic Bivalvia (Fiala-Médioni et al., 1986; Southward, 1986; Le Pennec et al., 1988; Herry et al., 1989), and in Oligochaetes (Giere and Langheld, 1987) living in reducing mud, as well as in Vestimentifera living near deep-sea hydrothermal vents (de Burg et al., 1989). In *L. pectinata*, the abundance of hemoglobin (Wittenberg, 1985; Kraus and Wittenberg, 1990) modifies the situation and the lysosome-like inclusions seem to be of two different types, one containing free heme, and the other being real lysosomes (Frenkiel et al., 1996).

In *L. pensylvanica*, each bacteriocyte contains typical peroxisomes. Such organelles have not been identified in the bacteriocytes of *C. orbicularis* and *L. pectinata*. Peroxisomes have already been described in the digestive diverticula of the bivalves *Mya arenaria* (Pal, 1971) and *Nucula sulcata* (Owen, 1972) but not in the gill cells of any bivalve examined so far. These organelles can be involved in the metabolism of carbohydrates and lipids or in glyoxalate cycle involving complex relationships between mitochondria and peroxisomes in gluconeogenic tissues such as the digestive diverticula of bivalves (Owen, 1972; Tolbert and Esser, 1981). Several hypotheses could be put forward. In the bacteriocytes of *L. pensylvanica*, a peroxisomal activity could protect the endosymbionts from the excess of intracellular oxygen which may lead to spontaneous oxidation of free sulfide or interfere with their autotrophic process. Another possibility is that the peroxisomes may carry out oxidization reactions which occur in the sulfur-oxidizing cycle. However, biochemical investigations will be necessary to check the role of these peroxisomes in the physiology of symbiosis.

In *L. pensylvanica*, smooth endoplasmic reticulum profiles are contiguous with the lateral and basal cytoplasmic membrane of bacteriocytes; similar relationships do exist in *L. pectinata* but have not been identified in *C. orbicularis*. Moreover, in *L. pensylvanica*, smooth endoplasmic reticulum profiles are frequently associated with peroxisomes. Such associations may be involved in energy transfer, in detoxification mechanisms or may constitute a calcium sequestering system. However, more investigation would be necessary to clarify the precise function of this smooth endoplasmic reticulum and of peroxisomes in bacteriocytes.

Sulfur-oxidizing eubacterial endosymbionts

In TEM views, the symbiont ultrastructure is quite similar in both host species even though *L. pensylvanica* symbionts appear to be larger than *C. orbicularis* symbionts. Both symbionts possess large, apparently-empty periplasmic vesicles. Such vesicles have been shown to contain sulfur which is dissolved during the dehydration procedure (Vetter, 1985). A lamellar annular structure which has been described in the gill bacterial endosymbionts of *C. orbicularis* (Frenkiel and Mouëza, 1995) but has not been identified in *L. pectinata* (Frenkiel et al., 1996) is frequently present in the bacterial endosymbionts of *L. pensylvanica*. The role of this structure, always located in the nuclear area of bacterial symbionts remains unknown. Durand et al. (1996) showed, by using 16S rRNA gene sequences, that *C. orbicularis* and *L. pensylvanica* seem to host the same species of intracellular gill symbiont. The symbiont population within the gills of both bivalves appears to be composed of a single species of bacteria. The fact that an identical bacterial species may establish symbiosis with two different host-species in similar sea-grass bed environments suggests that the endosymbiont transmission may occur by one of two ways proposed by Le Pennec et al. (1988) and Cary et al. (1993): (i) horizontal transmission which involves the spread of symbionts between contemporary hosts, or more likely (ii) environmental transmission which involves the reinfection of the new host-generation from an environmental stock of the free-living symbiont-form. However, the environmental transmission mode implies some complex recognition mechanisms between the bivalve host and the free-living symbiont-form, perhaps similar to those which occur between *Rhizobium* species and legume plants (Smith and Douglas, 1987; Pelmont, 1993).

Up to now, horizontal transmission has been reported in vestimentiferan species as *Riftia pachyptila* and *Ridgea piscesae* (Cary et al., 1993). Conversely, in bivalves, the vertical transmission through gametes has been recently demonstrated in *Bathymodiolus thermophilus* (Cary et al., 1993), in

three species of the genus *Calyptogena* (Cary and Giovannoni, 1993) as well as in *Solemya reidi* (Cary, 1994). Gros et al. (1996), using molecular probe technologies in association with experimental infestation, have demonstrated that the transmission mode of the *C. orbicularis*-endosymbiont is environmental. Preliminary results suggest that the transmission mode of *L. pensylvanica* symbiont is not vertical but probably also environmental. Research is in progress to confirm these results and to elucidate the transmission mode of the bacterial symbionts in the mangrove muddy bottom species, *L. pectinata*.

5. Conclusion

Although living in a similar environment, and hosting the same bacterial symbiont species, *Linga pensylvanica* exhibits structural and physiological features much different from those of *Codakia orbicularis*. Their most significant differences are the presence of peroxisomes in the bacteriocytes of *L. pensylvanica* which do not exist in *C. orbicularis*, the absence of granule-cells which are so abundant in *C. orbicularis*, and the complexity of the intercalary cells of *L. pensylvanica* unlike those found in *C. orbicularis*.

Each species has unique features which are likely to be determinant for their metabolic relationships with their symbionts:

- the presence of granule-cells with an abundant cystine-rich protein in *C. orbicularis*.
- the high level of cytoplasmic hemoglobin and the presence of a non-hemoglobin heme in peculiar microbodies in *L. pectinata*.
- the presence of peroxisomes in *L. pensylvanica*.

The comparison of the ultrastructural features of the gill filaments of *L. pensylvanica* with those of other Lucinidae – especially *C. orbicularis* and *L. pectinata* – raises some unresolved relevant questions: How the same bacterial species can establish symbiosis with two bivalve host-species which have such different structural and metabolic features? What is the transmission mode of these symbiotic bacteria?

We have to suppose that some of the morphological and physiological differences between the bivalve host-species are probably species-specific characteristics which may have evolved as adaptation to chemical parameters in their environment independently from the symbiosis. It should therefore be of outstanding interest to investigate the genetic proximity between species of Lucinidae and compare it with their symbiont genetic proximity. Only this genetic comparison could resolve the question of

phylogenetic relationships between the chemoautotrophic endosymbionts and their bivalve hosts.

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