Symbiotic bacteria of different species of genus *Oscarella* (Porifera, Homoscleromorpha, Plakinidae): comparative ultrastructural studies

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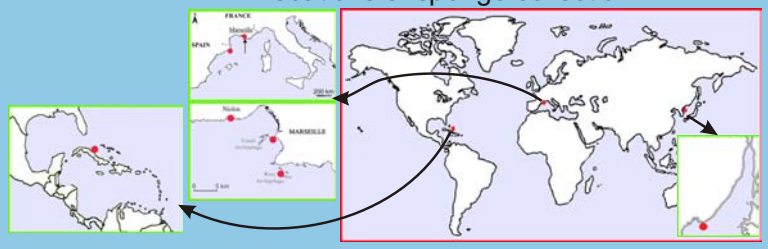
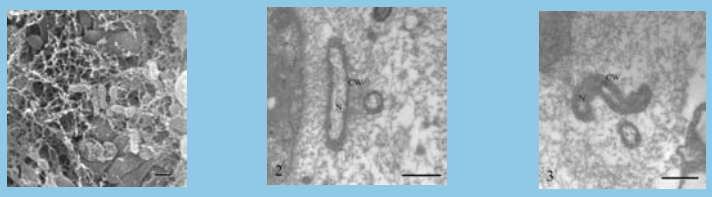
Symbiotic bacteria as an obligatory component of sponges can be helpful instrument for science investigation. We suggest using them in taxonomy of sponges without skeleton and for researches of coevolution events of these two organisms. The research objects are adult sponges, their embryos and larvae of genius *Oscarella* (Plakinidae, Homoscleromorpha). At present for their correct determination an ultrastructural data with description of symbiotic bacteria are widely used. Different species of *Oscarella* inhabiting together compact locations as caves can be hosts for different symbiotic bacteria. It is a convenient model for a comparative study of the host-symbiont system in related sponge species from different regions of the World Ocean.
The sponges were collected by SCUBA diving at depths of 5 - 25 m: *Oscarella tuberculata, O. lobularis, O. imperialis, O. microlobata* in the western Mediterranean Sea, *Oscarella* sp. 1 from western Japan Sea and *Oscarella* sp. 2 from Caribbean (north of Jamaica).

Locations of sponge collection



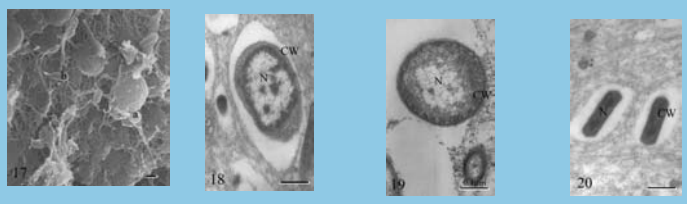
Symbionts of *Oscarella tuberculata* (Schmidt, 1868)

O .tuberculata has two morphotypes of symbionts (types *a* and *b*), which are in a close contact with mesohyl filaments. The first (type *a*) (Fig.1, 2) is rod-like about 1.5 µm in length and 0.3 µm in diameter with Gram-negative bacterial wall (CW), which, in most cases, is not good visible. A filamentous nucleoid (N) is surrounded by a thin layer of cytoplasm. The second (type *b*) (Fig. 3) is not numerous and has a spirilla-like body. The cell wall and the nucleoid are similar to the first type *a* except for a cytoplasmic layer that is thicker.



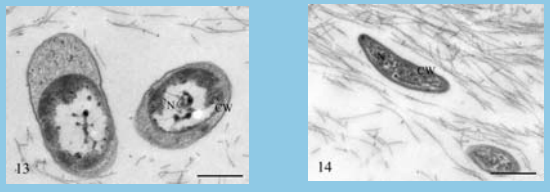
Symbionts of *Oscarella imperialis* Muricy et al. 1996

O. imperialis has three morphotypes of symbiotic bacteria (types *a*, *b*, and *c*). Type *a* is elliptical, 0.6 by 1.5 µm (Fig.17). A thin and dense layer of cytoplasm spreads under Gram-negative bacterial wall (CW). A nucleoid region (N) is filamentous. Symbionts of other morphological type *b* are 0.3 µm in diameter and about 1.5 µm in length (Fig. 18). Gram-negative cell wall (CW), a homogenous cytoplasmic layer of an average electron density and a filamentous nucleoid zone (N) are characteristics of these bacteria. Third variant of symbionts, type *c*, includes bacteria, which have a rod-like hexahedral shape 0.75 µm long by 0.2 µm wide (Fig.19). They differ from other bacteria by very electron-dense cytoplasm and a two-part nucleoid (N). The cell wall (CW) is Gram-negative.



Symbionts of *Oscarella* sp1

According to TEM data *Oscarella* sp.1 has at least two morphotypes of symbiotic bacteria (types *a* and *b*) (Fig. 13, 14). Type *a* is ovoid, whereas type *b* is elongated. The cell wall (CW) of both types consisting of two membranes (Gram-negative bacteria) has a very uneven thickness of a periplasmic space. A large field between cytoplasmic and outer membranes on one of cell poles suggests spirilla- or vibrion-like shape of these symbionts. Without SEM we can correctly estimate only a diameter of the cells, 0.8 µm and 0.25 µm, and approximate length, 1.8 µm and 1.1 µm for the bacteria of types *a* and *b*, correspondingly. In symbionts of type *a* a filamentous network of the nucleoid (N) is irregular with thick elements in center and thin filaments closer to periphery of the cell. Near the cytoplasmic membrane a small layer of granular cytoplasm occurs. In bacteria of type *b* the thick nucleoid filaments (N) form a more ordered voluminous structure with thin periphery of the dark cytoplasm.

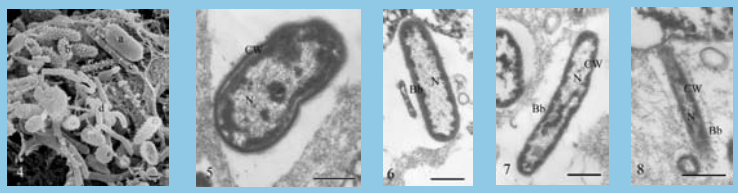


Resume

It is obviously that every *Oscarella* species in research collected from different places has a very distinctive set of symbiotic bacteria with specific traits. Thus symbiotic bacteria can be a good cytological marker for the sponges. Some problems in description of bacterial wall and determination of their taxonomic status can be resolved by FISH with oligonucleotides specific to various bacteria groups. Preliminary data allows suggesting an independent evolution within a very compact group of sponge species from family Plakinidae.

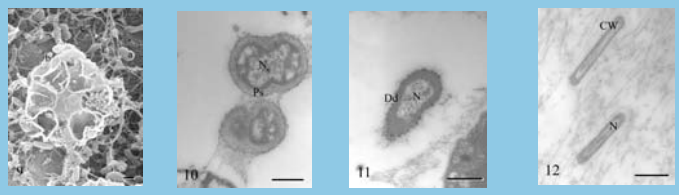
Symbionts of *Oscarella microlobata* Muricy et al. 1996

Symbiotic bacteria of *O. microlobata* are usually surrounded by the space without mesohyl filaments. They belong to 4 morphotypes (types *a*, *b*, *c*, and *d*) with different size characteristics and internal structure (Fig. 4). Largest bacteria (type *a*) (Fig.5) is oval, 1.2 µm in diameter and 3.2µm in length. The cell wall (CW) is the most probably Gram-negative with the periplasmic space of high electron density. Large volume of the nucleoid space includes a network of filaments (N) and electron-opaque bodies that are similar to peripheral cytoplasm. In some cases, there is a thin extracellular layer near the cell. The type *b* (Fig.6), 0.8 µm in diameter and 3.4µm in length, has a surprising cell wall appearance. A thick extracellular layer, which masks Gram-negative wall of bacteria, proves to be numerous blebs (Bb). Intracellular volume includes a spacious network of nucleoid filaments (N) and a small layer of the peripheral electron-opaque cytoplasm. Types *c* and *d* (Fig.7, 8) are rod-like with different sizes (0.4 and 0.2 µm in diameter and 4.0 and 2.8µm in length, correspondingly). An internal structure of their nucleoids(N) and of the cytoplasm is similar to type *b*. However, the Gram-negative cell wall (CW) carries solitary blebs (Bb).



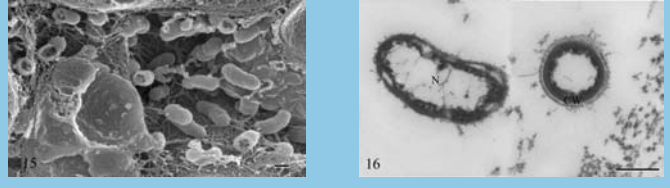
Symbionts of *Oscarella lobularis* (Schmidt, 1862)

Symbiotic bacteria of *O. lobularis* (types *a*, *b*, and *c*) have distinct characters but are difficult in definition of their type of bacterial wall. Bacteria of type *a* (Fig.9, 10) are ovoid, 0.8 µm in diameter and 1.5 µm in length. Cytoplasm consists of a central nucleoid zone with a network of filaments (N) and 1-2 dark granules, and a peripheral electron-opaque layer. Over an inner membrane there is a space (Ps) with short filaments, which have a radial arrangement. An outer membrane is not clearly visible. Therefore we cannot conclude precisely, either these bacteria have a Gram-negative cell wall with evidently a vast periplasmic space, or they are Gram-positive.
Symbionts of type *b* are rod-like about 2 µm in length and 0.4 µm in diameter (Fig.9, 11). Remarkable feature of these bacteria is dark dots (Dd) over the outer cell membrane. Probably, they are markers of a special interaction with filaments and cells of the mesohyl. The nucleiod (N) is filamentous. Both types of symbionts have a good contact with the filaments of mesohyl.
Symbiotic bacteria of type *c* are very small in diameter, 0.2 µm, and long, about 2 µm in length or more (Fig. 9, 12). Membranes of cell wall (CW) are tightly to each other. Nucleoid (N) looks like a filamentous network with a central narrow core.



Symbionts of *Oscarella* sp2

Symbionts of *Oscarella* sp.2 are rod-like slightly curved bacteria about 0.7 µm in diameter and 1.7µm in length (Fig. 15, 16). The cell wall (CW) consisting of two membranes and a narrow periplasmic space is obviously in contact with filaments of the mesohyl. Under the cell wall a cytoplasm looks like a dark irregular layer. Zone of the nucleoid (N) is clear with rare interrelating filaments.



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