In situ identification and localization of bacteria associated with Gyrodinium instriatum (Gymnodiniales, Dinophyceae) by electron and confocal microscopy

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The presence of intracellular bacteria in the dinoflagellate Gyrodinium instriatum Freudenthal & Lee has previously been described but the bacterial flora associated with this species has not been characterized. In this study, new results of transmission electron microscopy (TEM) and in situ hybridization using several bacterial group-specific oligonucleotide probes are presented. The long-term association of endocytoplasmic and endonuclear bacteria with G. instriatum has been confirmed. All endonuclear and most of the endocytoplasmic bacteria labelled were identified as belonging to the betaproteobacteria. Large clusters of Cytophaga-Flavobacterium-Bacteroides (CFB) were labelled and observed in the cytoplasm of the dinoflagellate cells, but were absent from the nucleus. Gammaproteobacteria were only observed outside the dinoflagellates. No alphaproteobacteria were detected either free-living or intracellular. Empirical observation of intracellular CFB reflected a degradation process of moribund dinoflagellate cells, whereas the systematic colonization of dinoflagellate nucleoplasm by betaproteobacteria suggested a true symbiotic relationship. Natural colonization may have occurred, perpetuated by vertical transmission of intracellular bacteria to the dinoflagellate daughter cells, via a pool of bacteria sequestered within the nucleus. Dividing bacteria were observed in the nucleus and equilibrium may be maintained by release of endonuclear bacteria to the cytoplasm through nuclear envelope constrictions.

Key words: confocal microscopy, dinoflagellates, Gyrodinium instriatum, in situ hybridization, intracellular bacteria, oligonucleotide probes, TEM, ultrastructure

Introduction

Bacteria and dinoflagellates are important members of the coastal plankton and their metabolism has a major input to pelagic energy flow and nutrient cycling through the water column (Cole, 1982; Doucette, 1995). Like other protozoa, both non-photosynthetic and photosynthetic dinoflagellates can actively prey on bacteria and other protozoa (Schnepf & Elbrächter, 1992; Jacobson & Anderson, 1996; Uchida et al., 1997; Skovgaard, 2000). Besides trophic interactions between the two organisms, bacteria and dinoflagellates can also develop close associations. The occurrence of bacteria-like particles within cells of cultured dinoflagellates was first described by Silva (1962). Subsequent transmission electron microscopy (TEM) studies provided further evidence of the presence of bacteria-like structures within several heterotrophic and photosynthetic dinoflagellate species (Gold & Pollingerh, 1971; Silva, 1978; Lucas, 1982; Rausch de Traubenberg et al., 1995; Doucette et al., 1998; Lewis et al., 2001). Generally these relationships have been postulated to be 'symbiotic' as the two organisms coexist without apparent symptoms and in some cases the association is maintained for a long time. Additionally, it has been suggested that bacteria associated with algal cells may play a direct or indirect role in phycotoxin production (Silva, 1982b; Kodama et al., 1990; Franca et al., 1995; Gallacher et al., 1997; Töbe et al., 2001).

Gyrodinium instriatum Freudenthal & Lee is an athecate photosynthetic dinoflagellate, which is often abundant in seawater and lagoons or inlets along the Portuguese coast. TEM observations of this species showed the occurrence of numerous intracellular bacteria both in the cytoplasm and in

* These two authors have contributed equally to this work.
Table 1. Oligonucleotide probes used

<table>
<thead>
<tr>
<th>Probe</th>
<th>Specificity</th>
<th>Sequence (5’-3’) of probe</th>
<th>Target site (rRNA positions)</th>
<th>% formamide in ISH buffer</th>
<th>HRP-labelled</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>EUB388R</td>
<td>Eubacteria</td>
<td>GCTGCTCCCGTGGAGGT</td>
<td>16S (338–355)</td>
<td>50</td>
<td>HRP</td>
<td>Amann et al. (1990)</td>
</tr>
<tr>
<td>ALF1b</td>
<td>Alphaproteobacteria</td>
<td>CGTTG(C/CTCGAGCCA</td>
<td>16S (20–35)</td>
<td>36</td>
<td>HRP</td>
<td>Modified from Manz et al. (1992)</td>
</tr>
<tr>
<td>ALF1b</td>
<td>Mitochondria that have one mismatch with ALF1b</td>
<td>CGTTG(A/CTCGAGCCA</td>
<td>16S (20–35)</td>
<td>36</td>
<td>–</td>
<td>Simon et al. (unpublished)</td>
</tr>
<tr>
<td>BET42a</td>
<td>Betaproteobacteria</td>
<td>GCCTTCCACCGCGTTTT</td>
<td>23S (1027–1043)</td>
<td>50</td>
<td>HRP or not when used as competitor</td>
<td>Manz et al. (1992)</td>
</tr>
<tr>
<td>GAM42a</td>
<td>Gammaproteobacteria</td>
<td>GCCTTCCACCGCGTTTT</td>
<td>23S (1027–1043)</td>
<td>50</td>
<td>HRP or not when used as competitor</td>
<td>Manz et al. (1992)</td>
</tr>
<tr>
<td>CF319a</td>
<td>Cytophaga-Flavobacterium</td>
<td>TGTTCCGTGTCCAGTTA</td>
<td>16S (319–336)</td>
<td>50</td>
<td>HRP</td>
<td>Manz et al. (1996)</td>
</tr>
</tbody>
</table>

HRP, horseradish peroxidase; ISH, in situ hybridization.

*Escherichia coli* numbering of the ribosomal RNA operon.

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Materials and methods

Culture conditions

The strain of *Gyrodinium instriatum* (LME 184, Lisbon, Portugal) used in this study was isolated in 1982 from Sto Andre's Lagoon, Portugal (Silva & Franca, 1985). The culture was maintained in a mixture of Provasoli's media ASP1 †ASP2 (1:1) (Provasoli, 1963) until 1998, when Guillard's medium f₂ was used (Sigma-Aldrich; Guillard & Ryther, 1962). The culture was maintained at 19–21 °C on a 14:10 light:dark cycle.

Cell fixation

Cells were typically harvested for fixation in mid- and late-exponential growth phase. For ultrastructural studies, a sample of the dinoflagellate culture was pre-fixed in 0.1% glutaraldehyde (Merck) for 1 h at room temperature. Cells were then concentrated by centrifugation and fixed for 1 h at room temperature with 4% paraformaldehyde (Merck), 3% glutaraldehyde (Karnowsky, 1965), and rinsed twice in 0–1 M PIPES buffer (piperazine-N,N'-bis[2-ethanosulphonic acid, Sigma-Aldrich) with 14% sucrose. The secondary fixative was made up of two solutions: (1) 1% OsO₄ (EMS) made up in water to which 3% potassium ferricyanide (Sigma-Aldrich) was added and (2) 6% sodium iodate (Sigma-Aldrich) made up in 0–1 M PIPES buffer. Equal volumes of the two solutions were added to the sample, which was then left at 4 °C for at least 1 h. Cells were then dehydrated in acetone (10%, 30%, and 60% for 30 min each; 90% for 1 h) and embedding in Durcupan ACM (Fluka). Sections were cut on a Reichert Ultracut E ultramicrotome, mounted on 150 mm grids, stained with uranyl acetate and lead citrate, and examined in a JEOL JEM100C electron microscope at 60 kV.

The aim of the present study was to localize and identify at the intracellular level the free and intracellular bacteria associated with a strain of *Gyrodinium instriatum* maintained in laboratory culture for 18 years. To further our understanding of the interactions between dinoflagellates and their associated bacteria, we used whole-cell hybridization of 16S rRNA-targeted oligonucleotide probes in conjunction with confocal microscopy to identify attached or intracellular bacteria within photosynthetic host cells. The major advantage of the latter technique was to allow rapid identification and threedimensional localization of bacteria within photosynthetic host cells.
were combined (A. Page, personal communication). Samples were left overnight at room temperature or at 4 °C. Cells were rinsed in water, dehydrated in an ethanol series and embedded in Spurr resin (Agar Scientific; Spurr, 1969).

For fluorescence in situ hybridization (FISH), cells were fixed with 1% paraformaldehyde in PBS buffer (phosphate-buffered saline) at 4 °C for 24 h, collected on inorganic filter membranes and dehydrated in an ethanol series (Amann, 1995). The filters were then stored in the dark at room temperature to await FISH experiments.

Probes
Oligonucleotide probes (listed in Table 1) were obtained from Interactiva (St. Malo, France) and labelled with HRP (Horseradish Peroxidase) (Roche Diagnostics) as described by Urdea et al. (1988) and Amann et al. (1992).

Fluorescence in situ hybridization
The protocol used for TSA-FISH (tyramide signal amplification–fluorescence in situ hybridization) was

Figs 1–6. Transmission electron micrographs of Gyrodinium instriatum. Fig. 1. Clusters of bacteria (arrows) in the cytoplasm (C) and dividing bacteria (arrowhead). Fig. 2. Intranuclear bacterial clusters in the nucleus (N) periphery. Note isolated bacteria (arrow) among the chromosomes (Chr). Fig. 3. DNA fibrils (arrow) extending from the dinoflagellate chromosome (Chr) to the bacteria (Ba). Fig. 4. Bacteria being released from the nucleus (N) to the cytoplasm (C) (arrow). Fig. 5. Dividing bacteria (arrowhead) inside the nucleus (N). Fig. 6. Degrading bacteria (arrow) in cytoplasmic vacuole. Scale bars represent 1 µm.
according to Biegala et al. (2002). The hybridized cells immobilized on filters were kept at 4 °C in the dark. Confocal observations were made within 2 weeks of preparation without significant loss of fluorescence.

Microscopy

Ultrathin sections were cut on an LKB 8800 Ultratome III microtome (Bromma, Sweden) with a Diatome diamond knife (Bienne, Switzerland) and counterstained with uranyl acetate and lead citrate (Reynolds, 1965). Stained grids were examined on a Philips EM 301 TEM (Eindhoven, The Netherlands).

Fluorescence images were acquired with a confocal laser scanning microscope (CLSM) Fluview (Olympus Optical, Tokyo, Japan) equipped with an argon–krypton laser (643-OLYM-A03 Omichrome, Melles Griot, Carlsbad, CA, USA) and a pulsed laser (Mira 900, Coherent, Santa Clara, CA, USA).

Results

TEM

TEM observations revealed the presence of numerous intracellular bacteria in the majority of the cell sections, both in the cytoplasm (Fig. 1) and in the nucleus (Figs 2, 3). Endonuclear bacterial structures were observed dispersed throughout the nucleoplasm, aggregated in dense clusters, less frequently isolated between the chromosomes and usually surrounded by a transparent halo (Fig. 2). Endonuclear bacteria were mainly observed at the periphery of the nucleus adjacent to the nuclear envelope, while the chromosomes were aggregated in a more central region of the nucleus (Fig. 2). Few bacteria established close connections with the chromosomes. When such cases occurred DNA fibrils could be seen extended towards the bacteria through an electron-transparent zone (Fig. 3). Occasionally, invaginations of the nuclear envelope were observed enclosing single bacteria or clusters of bacteria that appeared to be about to be released into the cytoplasm by constriction of the nuclear membrane (Fig. 4). Actively dividing bacteria were observed in the nucleus (Fig. 5) and more rarely in the cytoplasm (Fig. 1). Bacteria in the cytoplasm were organized in clusters surrounded by a host membrane or, alternatively, isolated among the dinoflagellate organelles and separated from them by a transparent region (Fig. 1). Furthermore, some sections showed the presence of cytoplasmic vacuoles containing electron-dense masses and concentric multilamellar structures (Fig. 6).

Fluorescence in situ hybridization

Endocytoplasmic and endonucleoplasmic bacteria were successfully labelled with the EUB338R probe (specific for the Bacteria domain) in all Gymnodinium instriatum cells examined (Fig. 7). Hybridized bacteria were abundant in the nucleus and cytoplasm and were present either singly or in clusters. Bacteria present in dividing dinoflagellates were also strongly labelled with this probe (Fig. 8a). Hybridization with probes (Table 1) specific for gram-negative alpha-, beta- and gammaproteobacteria and Cytophaga-Flavobacterium-Bacteroides (CFB) showed that endonucleoplasmic and endocytoplasmic bacteria were mainly labelled by the BET42a probe (Figs 9a, 10a, 11a, 12a). Endonuclear betaproteobacteria were mostly observed at the nuclear periphery, although a few were located between the dinoflagellate chromosomes (Fig. 10a). No intracellular alpha- and gammaproteobacteria were detected in any of the cells examined (Figs 9a, 11a). Some dinoflagellates showed intense endocytoplasmic fluorescence when hybridized with the probe targeting CFB, although no endonuclear labelling was observed. Bacteria labelled with the CF319a probe were associated in large clusters (15 × 5 µm; Fig. 12a). All extracellular bacteria were labelled with the EUB338R probe (data not shown), and most belonged to the beta- and gammaproteobacteria. Very few extracellular bacteria were labelled with the CF319a probe and none were labelled with the alphaproteobacteria.

Discussion

The presence of intracellular bacteria in G. instriatum has been described at a morphological level in numerous studies (Silva, 1982a, b; Silva & Franca, 1985). However, the present work identifies for the first time the bacterial flora associated with this dinoflagellate using whole-cell hybridization of 16S rRNA oligonucleotide probes. Among the different classes of eubacteria probed, representatives of betaproteobacteria were found to be the dominant microorganisms intracellularly and extracellularly. Furthermore, they were the only group of bacteria identified within the nucleoplasm of G. instriatum. This result is surprising as endosymbiotic bacteria of eukaryotic organisms usually belong to the alpha and gammaproteobacteria (Garrity, 2001). Nevertheless, symbiotic relationships of betaproteobacteria with some groups of insects have been described by Du et al. (1994), Fukatsu & Nikoh (2000) and Von Dohenl et al. (2001). To our knowledge, the present study reports for the first time the presence of intracellular betaproteobacteria in dinoflagellates or other free-living protists (Fokin et al., 1996; Fritsche et al., 1999; Seibold et al., 2001; Schweikert & Meyer, 2001). Betaproteobacteria have been considered rare in marine ecosystems but dominate freshwater environments (Glöckner et al., 1999). Nevertheless, betaproteobacteria have recently been observed at high concentrations in marine sediments (Nold et al., 2000).
Bacteria associated with G. instriatum

Figs 7–12. Confocal laser scanning microscope 0.7 μm optical sections through Gyrodinium instriatum cells immobilized on a filter. Figs 7a, 8a, 9a, 10a, 11a, 12a excited at 488 nm and hybridized with specific probes (Table 1) labelled with fluorescein using TSA-FISH technique. Figs 7b, 8b, 9b, 10b, 11b, 12b excited at 380 nm and stained with DAPI. Fig. 7a. Intracellular bacteria in the cytoplasm (C) and in the nucleus (N) labelled with the EUB338-HRP probe, present both isolated and in clusters. Fig. 8a. Dividing cell showing the same distribution of labelled intracellular eubacteria as observed in Fig. 7a. Fig. 9a. Optical section of a cell with no intracellular bacteria labelled with the ALF1b-HRP probe. Fig. 10a. Three dinoflagellate cells with endocytoplasmic and endonuclear bacteria (arrows) labelled with the BET42a-HRP probe. Fig. 11a. Optical section of two dinoflagellates with no intracellular bacteria labelled with GAM42a probe. Fig. 12a. Large clusters of endocytoplasmic bacteria labelled with the CF319a-HRP probe. Scale bars represent 20 μm.

Marine bacterioplankton is dominated by alphaproteobacteria and gammaproteobacteria as well as CFB (Glöckner et al., 1999; Cottrell & Kirchman, 2000; Hagström et al., 2000; Riemann et al., 2000), which occur either as free-living organisms or attached to phytoplankton. These three classes of bacteria have already been found associated with different species of dinoflagellates (Lafay et al., 1995; Hold et al., 2001a). A recent study (Hold et al., 2001b) indicated that the majority of the bacteria associated with four different dinoflagellate clonal cultures were alphaproteobacteria, and fluctuations in abundance were observed at different phases of the growth cycle. The absence of alphaproteobacteria either in the culture medium or associated with this clone of G. instriatum is therefore surprising. There are several possible explanations for this discrepancy. Firstly the results produced in the present study may reflect the bacterial community present at the time of sampling for hybridization experiments. Secondly, the dominance of alphaproteobacteria in dinoflagellate cultures, as previously mentioned by other authors, might not reflect their real abundance in nature. All these studies used polymerase chain reaction (PCR)-based techniques, which are known to preferentially amplify a particular genus or group of target organisms. And finally, in natural environments alphaproteobacteria have been described attached to live or detrital particles less frequently than CFB and gammaproteobacteria (Riemann et al., 2000; Cottrell & Kirchman, 2000). It is thus probable that when G. instriatum cells were isolated for the establishment of this clonal culture, no alphaproteobacteria were attached to the dinoflagellates. In contrast, CFB, gamma and betaproteobacteria may have been recovered from nature in the clonal culture.

In a few G. instriatum cells, strong cytoplasmic colonization by CFB was observed. Mixotrophy is a nutritional strategy quite common among different
dinoflagellate species (Hansen, 1991; Schnepf & Elbrächter, 1992; Jacobson & Anderson, 1996; Skovgaard, 2000), including *G. instriatum* (Uchida et al., 1997). Therefore, it is possible that the observed cytoplasmic CFB were taken up as prey and maintained as isolated clusters in the cytoplasm. However, these bacteria were rare as free-living organisms in the culture medium, making this hypothesis unlikely. Additionally, the FISH signal observed was very strong, which would not be the case if the target cells were being digested. It seems more reasonable to assume that the large CFB clusters observed corresponded to colonization of senescent or dead dinoflagellate cells by this group of bacteria. This bacterial strategy is common within the CFB group, which have been shown to develop after the collapse of a phytoplankton bloom (Riemann et al., 2000). Additionally, from pure-culture studies, it is known that members of this class are able to aerobically degrade a large spectrum of substrates ranging from various proteins, carbohydrates, pesticides and insecticides to complex macromolecules (Bernardet et al., 1996). Although the latter hypothesis is the most probable, an intimate association between these two organisms, such as a symbiosis or parasitism, cannot be ruled out. It has recently been reported that members of the CFB group establish symbiotic relationships with other protozoa such as amoebae (Horn et al., 2001). The nature of the relationship between *G. instriatum* cells and bacteria from the CFB is unknown, but it seems quite different from the one established with betaproteobacteria, both in the number of dinoflagellate cells colonized (it represents only 2% of the observed cells versus 100% of cells with betaproteobacteria) and in the intracellular location of the two bacterial types.

The presence of endonuclear bacteria in dinoflagellates is rare, and to our knowledge has been reported in a limited number of species (Silva, 1978; Silva & Franca, 1985). The origin of the endonuclear betaproteobacteria in *G. instriatum* is not fully understood. It can be hypothesized that under certain environmental conditions, such as low nutrient levels, free-living bacteria may be taken up as prey, with those colonizing the nucleus avoiding digestion in the cytoplasm. This route of nuclear infection has been previously suggested by Görtz (1986) to explain the origin of endonucleobiosis in ciliates. Nuclear colonization by prokaryotic microbes is commonly observed in protozoa such as euglenoids (Leedale, 1969) and ciliates (Görtz et al., 1989; Kawai & Fujishima, 2000). Intranuclear bacteria may have advantages over those that colonize the cytoplasm because: (1) they ensure stability of the association by homogeneous distribution to daughter cells during cell division – in *G. instriatum* the nuclear envelope remains intact during mitosis and (2) nuclear bacteria could benefit from the nuclear pools of metabolites, renewed at each host division (Görtz, 1986).

TEM observations showed dividing bacteria within the nucleus of dinoflagellate cells (Fig. 5), without apparent prejudice to the host’s normal growth, which is similar to the growth rate of clonal cultures without endocellular bacteria (data not shown). Similar TEM observations have been made on this species by Silva & Franca (1985), who suggested the maintenance of a stable equilibrium between the two organisms. As described by these authors, a decrease in the host’s division rates may lead to an increase in intranuclear bacteria, some of which are subsequently expelled into the cytoplasm via vesicles pinched off from the nuclear membrane, as a reaction of the host. Supporting this hypothesis are TEM observations showing nuclear envelope constrictions enclosing bacteria (Silva & Franca 1985, figures therein and Fig. 4 in this study), indicating that the nucleus in this *G. instriatum* clone may act as a reservoir of bacteria which will be either ‘expelled’ or digested in the cytoplasm according to the host’s needs (Silva, 1982b).

Some endonuclear bacteria were observed dispersed among or closely apposed to dinoflagellate chromosomes through host DNA fibrils. Similar observations have been made in ciliates, and it has been suggested that certain bacterial endonucleobionts digest host chromatin (Görtz, 1986). Görtz further suggested that in a densely colonized nucleus, the destruction of the chromatin would be significant and probably fatal to the host. The *G. instriatum* clone under study has been successfully maintained in culture without loss of its endonuclear bacteria for a long period. Therefore, it seems unlikely that *G. instriatum* possesses endonuclear bacteria that feed directly on chromatin, unless it has an adequate chromatin repair mechanism ensuring cell viability.

The transfer of prokaryotic DNA to a eukaryote has been previously reported (Taylor, 1979). The presence of abundant intranuclear bacteria and the close connection established by some with dinoflagellate chromosomes may lead to speculation about an eventual uptake of prokaryotic DNA, or its export and translation by the host. Besides its evolutionary implications, it could act as a mechanism through which endonuclear bacteria induce the production of special metabolites by the host cell. This aspect may be of particular relevance in harmful algal species.

The microscopic study of *Gyrodinium instriatum* cells confirmed the maintenance of the association of this dinoflagellate with intracellular bacteria over a period of 18 years. As the cells were not cultured axenically, the possibility exists that during this period bacteria were taken up and released from/
into the medium. However, the long-term presence of intranuclear bacteria suggests a symbiotic relation between the dinoflagellates and these bacteria.

Further studies on interactions between dinoflagellates and bacteria are necessary to improve understanding of the way both organisms survive and benefit from the association. Since one of the sources of genetic novelty has been identified as being the repeated fusion of bacterial endosymbionts with host cells (Taylor, 1979), these studies may help to give a better insight into evolutionary processes in the Dinophyceae. Sequencing of bacterial ribosomal genes and the design of probes should be pursued and applied to both cultured and natural samples, thereby enlarging the limited information available about the natural bacterial flora associated with dinoflagellates. Although toxicity tests were not part of this study, this species has previously been described as being toxic (Silva, 1982b). Therefore, an approach combining the techniques used here with toxin detection testing could add much relevant information regarding the involvement of bacteria in the phenomenon of harmful algal blooms (HAB).

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References


ties associated with toxic and non-toxic dinoflagellates: Alexander-
drium spp. and Scrippsiella trochoidea. FEMS Microbiol. Ecol.,

HORN, M., HARZENETTER, M.D., LENNER, T., SCHMID, E.N.,
Cytophaga-Flavobacterium-Bacteroides phylum as intracellular

JACOBSON, D. & ANDERSON, D. (1996). Widespread phagocytosis of
ciliates and other protists by marine mixotrophic and hetero-

137A.

KAWAI, M. & FUISHIMA, M. (2000). Invasion of the macronucleus of
Paramaecium caudatum by the bacterium Holospora obtusa:
fates of the bacteria and timings of invasion steps. Eur. J.
Protistol., 36: 46–52.

KODAMA, M., OGATA, T., SAKAMOTO, S., HONDA, T. & MIWATANI,
T. (1990). Production of paralytic shellfish toxins by a bacterium
Moraxella sp. isolated from Protogonyaulax tamarensis. Toxicon,
28: 707–714.

LAFAY, B., RUMY, R., RAUSCH DE TRAUENBERG, C., BREITTMAYER,
nov., a new marine bacterium isolated from the phytoplankton of
the toxic-producing dinoflagellate Proorocentrum lima. Int. J. Syst.


Bacterium–dinoflagellate interactions: investigative microscopy
of Alexandrium spp. (Gonyaulacales, Dinophyceae). Phycologia,

LUCAS, I.A.N. (1982). Observations on Noctiluca scintillans Macart-
ney (Ehrenb.) (Dinophyceae) with notes on an intracellular bacterium.

MANZ, W., AMANN, R., LUDWIG, W., WAGNER, M. & SCHLEIFER,
major subclasses of proteobacteria: problems and solutions. Syst.

MANZ, W., AMANN, R., LUDWIG, W., VANCANNEY, M. & SCHLEIF-
oligonucleotide probes designed to investigate bacteria of the
phylum Cytophaga-Flavobacter-Bacteroides in the natural envi-

Northwest marine sediments contain ammonia-oxidizing bacteria
in the β subdivision of the Proteobacteria. Appl. Environ.


RAUSCH DE TRAUENBERG, C., GERAUD, M.-L., SOYER-GOBILLARD,
trum lima and its associated bacteria. Eur. J. Protistol., 31:
318–326.

REYNOLDS, E.S. (1963). The use of lead citrate at high pH as an
electron-opaque stain in electron microscopy. J. Cell Biol., 17:
208–212.

RIEMANN, L., STEWART, G. & AZAM, F. (2000). Dynamics of
bacterial community composition and activity during a mesocosm

dinoflagellates. A review with emphasis on cell biological aspects.

SCHWEIKERT, M. & MEYER, B. (2001). Characterization of in-
tracellular bacteria in the freshwater dinoflagellate Peridinium

endocytic bacteria in the dinoflagellate Noctiluca scintillans.

SILVA, E.S. (1962). Some observations on marine dinoflagellate
cultures. III. Gonyaulax spinifera (Clap. and Lach.) Dies.,
Gonyaulax tamarensis Leb., and Peridinium trochoideum (Stein)

SILVA, E.S. (1978). Endonuclear bacteria in two species of dino-

SILVA, E.S. (1982a). Relationship between dinoflagellates and
intracellular bacteria. In Marine Algae in Pharmaceutical
de Gruyter, Berlin.

SILVA, E.S. (1982b). Toxic clones of Gyrodinium instriatum with
endonuclear bacteria. Proc. 8th Int. IUPAC Symposium on
Mycoxins and Phycotoxins, Vienna, Austria, 216–219.

SILVA, E.S. & FRANCA, S. (1985). The association dinoflagellate-
bacteria: their ultrastructural relationship in two species of dino-

in the plastidic dinoflagellate Gyrodinium resplendens (Dino-


evolutionary impact of intracellular symbioses. Proc. R.

TOBE, K., FERGUSON, C., KELLY, M., GALLACHER, S. & MEDLIN,
L.K. (2001). Seasonal occurrence at a Scottish PSP monitoring
site of purportedly toxic bacteria originally isolated from the
toxic dinoflagellate genus Alexandrium. Eur. J. Phycol., 36:
243–256.

a photosynthetic dinoflagellate Gyrodinium instriatum on lori-

URDEA, M.S., WARNER, B.D., RUNNING, J.A., STIMPEN, M., CLYNE,
hybridization assay methods using fluorescent, chemiluminescent
and enzyme labeled synthetic oligodeoxiribonucleotide probes.

VON DOHLEN, C.D., KOHLER, S., ALSOP, S.T. & MCMANUS, R.
(2001). Mealybug β-proteobacterial endosymbionts contain