Protistology 3 (2), 138-144 (2003)

Protistology

Cell wall ultrastructure and intracytoplasmic bacteria in hypnocysts of toxic *Alexandrium tamarense* (Dinophyceae)

Michael Schweikert

Department of Zoology, Biological Institute, University of Stuttgart, Stuttgart, Germany

Summary

Hypnocysts of toxic *Alexandrium tamarense* (Lebour) Balech collected during a bloom in the North Sea near Scotland (UK) have been investigated ultrastructurally. Details of cell wall morphology and a cell wall covering, not mentioned previously in the literature, are given. The presence of intracellular bacteria has been shown by means of transmission electron microscopy. These bacteria were present in early and mature hypnocysts, but absent in flagellated vegetative cells found in the same region. The evidence for the transmission of toxic bacteria via hypnocysts and their inoculation into the environment by excysted dinoflagellates is discussed.

Key words: *Alexandrium tamarense*, harmful algal blooms, hypnocysts, intracellular bacteria, ultrastructure.

Introduction

Alexandrium tamarense (Lebour, 1925) Balech (Dinophyceae) is known to produce potent neurotoxins causing paralytic shellfish poisoning (PSP) and is therefore of worldwide scientific interest. The life cycle of *A. tamarense* resembles a typical dinoflagellate development (e.g., von Stosch, 1965, 1973). Vegetative cells multiply by mitosis and can form haploid pellicle cysts. The sexual reproduction results in a zygotic resting stage named the hypnocyst (e.g., Fritz et al., 1989; Anderson et al., 1996). The hypnocysts settle down into the sediment where they can stay dormant for several years, until the excystment of a zygote gives rise to haploid vegetative cells. Hypnocysts can form seedbeds (e.g., Anderson et al., 1982, 1983) inoculating new vegetative *A. tamarense* cells seasonally into the water body. They can strongly impact the ecosystem and cause heavy losses to local industry (e.g., fisheries, aquaculture and tourism) when reaching high cell densities (Rosenberg et al., 1988; Franca, 1991; Penna et al., 1993; Arzul et al., 1995; Delgado et al., 1997). The toxicity of hypnocysts of *A. tamarense* was investigated by Oshima et al. (1992) who reported high paralytic shellfish toxin values. The ultrastructure of dinoflagellate hypnocysts was investigated mainly by light microscopy, rarely ultrastructurally, possibly because of difficulties in the preservation of samples for transmission electron microscopy. Most investigations of dinoflagellate hypnocysts were made by paleontologists dealing with fossilized and generally calcareous cysts. Information on mating, generation of zygotes and cyst formation of *Gonyaulax tamarensis*

(syn. *A. tamarense*) was given in Turpin et al. (1978). Extracellular (Lafay et al., 1995; Prokic et al., 1998) and intracellular bacteria are discussed in the context of PSP events (Silva, 1982, 1990; Gallagher, 1997). Clear morphological evidence for intracellular bacteria in environmental samples of cells from the dinoflagellate genus *Alexandrium* has not been given so far. Ultrastructural investigations of *Alexandrium* sp. revealed bacteria-like structures in cells at nearly all stages of their life cycle except hypnocysts (Lewis et al., 2001). The bacteria were not found in the same strain by fluorescence in situ hybridization with phylogenetic oligonucleotide probes specific for eubacteria (Biegala et al., 2002). It is still unclear what the source of the toxic compounds is: dinoflagellates themselves, intracellular bacteria, or the symbiotic association. Toxicity was associated with dinoflagellates (e.g., Oshima et al., 1992), but toxin-producing bacteria were also found (Kodama et al., 1988; Kodama et al., 1990; Silva, 1990; Gallagher et al., 1997; Kopp et al., 1997; Lu et al., 2000). This article presents evidence for intracytoplasmic bacteria in hypnocysts of *A. tamarense* and gives new ultrastructural details of cell wall morphology of early and mature hypnocysts of *Alexandrium tamarense*.

Material and methods

Plankton net samples were taken on board of the research vessel FS HEINCKE (cruise HE-105) during a bloom of *Alexandrium tamarense* (Lebour) Balech in the Firth of Forth, the North Sea near Scotland (UK) in May 1998. Cells were kept in 50 ml cell culture flasks (Falcon) at about 18°C under day light in the laboratory on board. After generation of hypnocysts they were kept under these conditions until their transfer into the laboratory in Stuttgart.

Light micrographs were made on board using an Olympus BH2 microscope, equipped with a camera and an AGFA-Ortho 25 B/W film, under differential interference contrast conditions.

Scanning electron microscopy was performed at the BAH/AWI Wattenmeerstation in List/Sylt. Cysts were fixed in 2% glutardialdehyde in sea water for 15 min and were filtered onto a 0.2 µm Nucleopore PC-membrane filter (Fa. Millipore). For dehydration 30% ethanol was used for a minimum of 15 min. Cells were transferred into acidic dimetholpropane (DMP) and finally in pure DMP for 15 min, respectively. Critical point drying was performed using a CPD 020 (Balzers Union) with liquid CO_2. Filters were mounted on a specimen holder and dried at 30°C in a dry chamber. Prepared cells were sputtered with gold-palladium in a SCD Sputter Coater (BAL-TEC). Micrographs and ultrastructural investigations were done using a Zeiss DMS 980A Scanning Electron Microscope at 15kV equipped with a Kontax camera and an Ilford FP4 Plus B/W film.

For transmission electron microscopy single cysts were embedded in low melting sea prep agarose (Reize and Melkonian, 1989), fixed in 2% glutardialdehyde in culture medium for 1h and postfixed in 1% OsO_4 in culture medium for 1h at room temperature. Cells were dehydrated in an acetone series and embedded in Spurr's resin (Spurr, 1969). Ultrathin sectiones were made using a Leica UCT and poststained with 1% aqueous uranylacetate and lead citrate (after Reynolds, 1963) before viewing in a Zeiss EM 10 at 60 kV.

Results

Encystment of most of the isolated *A. tamarense* cells occurred within 6 hours after transfer into culture medium. Cysts were identified as hypnocysts of *A. tamarense* (syn.: *Gonyaulax tamarensis* Lebour 1925, *Protogonyaulax tamarensis* (Lebour)) by light microscopy (Fig. 1 A). Hypnocysts isolated from the North Sea near Scotland (UK) are elongate-cylindrical cells with rounded ends about 70 µm long and about 30-40 µm wide (Figs 1 A, B). Every cell was surrounded by a prominent, colorless cell wall, about 1-2 µm thick (Fig. 1 A). The cyst wall was covered by a transparent sticky layer (Fig. 1 A) to which particles like bacteria, diatoms and sand grains adhered (Figs 1 A, B). Cyst content determined the characteristic appearance of *A. tamarense* cysts. Distinctive features like starch grains, lipid globules, crystalline structures, nucleus and a characteristic accumulation body pigmented orange-red to brown were present (Fig. 1 A).

Ultrastructural investigation of mature hypnocysts of *A. tamarense* generally reveals the same characteristics as the light microscopy (Fig. 1 C). The cyst contents resemble those of a typical phototrophic dinoflagellate, with dinokaryon, chloroplasts, starch grains, an accumulation body and intracellular crystals distributed at both rounded ends of the cyst (Fig 1 C). In addition, the cytoplasm of a mature cyst contains intracytoplasmic bacteria about 0.8 µm in diameter, irregular in shape, but clearly distinguishable from mitochondria by their size and the presence of a bacterial cell wall (Fig. 1 D). Median sections reveal morphological details of the hypnocyst cell wall (Fig. 1 E). The rigid part of the cell wall is composed of six layers. Additionally to these layers the wall is covered with a layer of filaments arising from a basal layer connecting the filaments to each other. The innermost structure, close to the plasma membrane, a fibrillar component of the cell wall about 0.8 µm thick, with

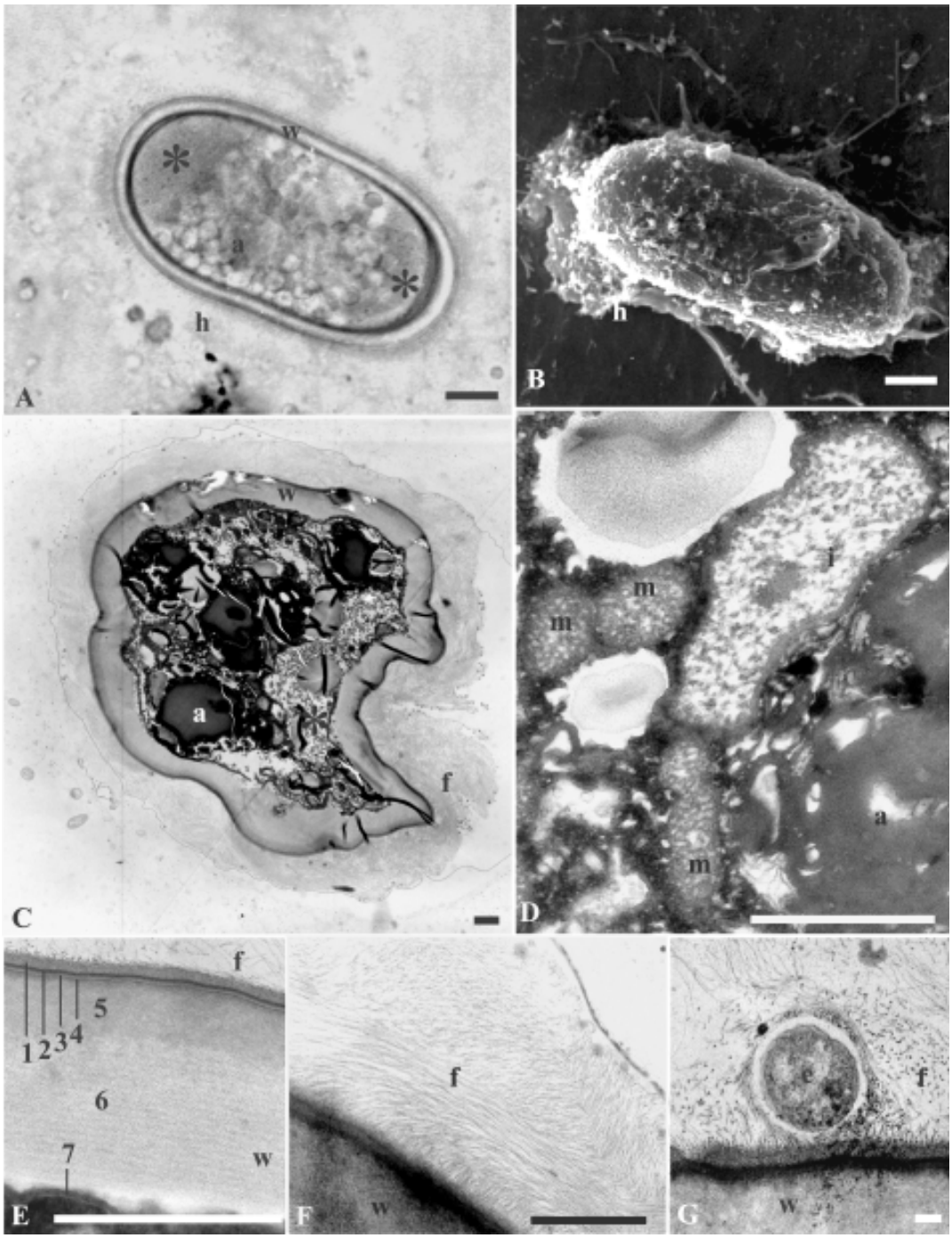

Fig. 1. Hypnocysts of *Alexandrium tamarense*. A - Light micrograph of a hypnocysts of *A. tamarense*. B - Scanning electron micrograph of a hypnocyst of *A. tamarense*. C - mature hypnocyst. D - intracellular bacterium in mature hypnocyst. E - Morphological details of the hypnocyst cell wall. F - Details of the filamentous coating of the hypnocyst cell wall. G - Extracellular bacterium invading the filamentous coating of a mature hypnocyst. Abbreviations: a - accumulation body, e - extracellular bacteria, f - extracellular filaments, i - intracellular bacterium, m - mitochondium, w - cell wall, asterisk - crystals, 1 - final unordered layer with emerging filaments, 2 - electron-dense layer, 3 - very electron-opaque layer resembling the sporopollenin layer, 4 - very thin electron-dense layer, 5 - more electron-dense unordered fibrillar layer, 6 - fibrillar striated part of the cell wall, 7 - plasma membrane. Scale bars: A, B - 10μm; C, D, G - 1μm; E, F - 0,1μm.

the characteristic appearance of cellulose, is observed (Fig. 1 E). A electron-dense layer with different orientation of the fibrils, about 0.2 µm thick follows, covered with a delicate, more electron-dense layer (Fig. 1 E). This layer is followed by an electron-transparent layer and again by a very thin electron-dense layer. The cyst wall is covered with filamentous structures (Figs 1 C, E -G) arising from the final thin layer of the cyst wall (Fig. 1 E). The filaments are almost of the same length (about 5 µm) overall the cyst surface but the thickness of this layer depends on the orientation of the filaments. Filaments are not agglutinated to each other, even when they are distorted (Figs 1 C, E -G). Some extracellular bacteria invade this filament layer until the basal part of the filaments (Fig. 1 G), whereas other particles remain superficially attached. The filaments around the bacteria appear to be agglutinated (Fig. 1 G).

Early hypnocysts are surrounded by a thin cell wall less than 1µm thick (Fig. 2 A) and their cytoplasm is not concentrated in a centrally positioned cyst area, like in mature cysts. The cell contents of early hypnocysts resemble those of vegetative cells, while mature hypnocysts are more dehydrated. Extracellular and intracellular bacteria are also present in these early hypnocysts (Figs 2 A, B). Intracytoplasmic bacteria lie in the cytoplasm not surrounded by a peribacterial membrane. These bacteria are of irregular shape, about 1.5 µm in diameter (Fig. 2 B). They are covered with a fuzzy electron-opaque layer (Fig. 2 B) and surrounded by a cytoplasm-free halo.

Discussion

Toxicity of *Alexandrium tamarense* cells from the same samples was confirmed directly at the sampling site on board of the RV HEINCKE at the east coast of Scotland by HPLC and by a fast fluorimetric assay (FFA) (Gerdts et al., 2002). Cysts were identified by light microscopy as hypnocysts of *A. tamarense* (Anderson and Wall, 1978; Turpin et al., 1978; Fritz et al., 1989; Thomas, 1997; Wall, 1975, Fukuyo et al., 1990).

Encystment of most cells occurred rapidly after transfer into culture. Generally, zygotes of *A. tamarense* in culture need up to several weeks to form cysts (e.g., Turpin et al., 1978). A rapid encystment of the majority of *Alexandrium tamarense* cells indicated that this bloom consisted mainly of zygotes. *A. tamarense* in the bloom waters investigated around Scotland can originate from hypnocysts of local sediments or can be transported by global water currents from another area of the ocean where the bloom started (Medlin et al., 1998).

Ultrastructural investigations revealed the presence of intracellular bacteria in early and mature cysts. However, they were absent in vegetative cells from the same area at the same time. An infection or uptake during encystment is possible. It is not clear whether this was a symbiotic or a parasitic infection. The bacteria were found in the cytoplasm, not surrounded by a peribacterial membrane, which supports the idea that they do not serve as food organisms. The fact that the cytoplasm changes its appearance during encystment has already been described for other dinoflagellates (Fritz et al., 1989; Gao et al., 1989). In *A. tamarense* the cytoplasm becomes concentrated in the centre of the cyst while at both ends crystals appear. As a consequence, intracellular bacteria change their appearance and size during encystment of the dinoflagellate. This is most probably a result of concentration of cytoplasmic contents due to the dehydration during encystment. An accumulation body (Taylor, 1968) is generated, displaying an orange-red to brown area, sometimes mentioned as characteristic of *A. tamarense* cysts. Differences in size and shape between the intracellular bacteria detected and mitochondria are evident. The bacterial cell wall is clearly visible in ultrathin sections. The role of intracellular bacteria in the production of PSP-toxins is still obscure, but the presence of intracellular bacteria in a PSP-producing dinoflagellate provides new data relevant to this problem. Furthermore, the presence of intracellular bacteria in hypnocysts points to the cysts' potential to harbour bacteria for a long time, probably inoculating this symbiosis or the bacteria separately into the water body after excystment. If a long-term association is formed, a reinfection of the dinoflagellate with free-living toxin-producing bacteria after excystment is not essential for the installation of this symbiosis. Although a parasitic infection cannot be ruled out, the persistence of these bacteria inside the hypnocyst until the germination of the dinoflagellate is more likely, since morphological adaptations to dormancy in these intracytoplasmic bacteria are also obvious.

Mature hypnocysts are surrounded by a transparent sticky layer previously described as mucilage. Bacteria and other particles adhere to this layer, which probably increases sedimentation and supports the persistence in the sediment. Transmission electron microscopy has revealed some details of the hypnocyst morphology, not mentioned in earlier publications. The composition of the cyst wall is complex. All in all six layers can be discerned covered with an additional layer of extra-cellular filaments, that has been not described ultrastructurally in dinoflagellate cysts so far. The first two layers, close to the plasma membrane, appear ultrastructurally similar to cellulose. The electron-opaque final layer of the cyst is most probably a sporopollenin layer (Brooks, 1971; Anderson and Wall, 1978). The cyst wall surface is covered by long filaments arising out of an electron-dense basal layer separated

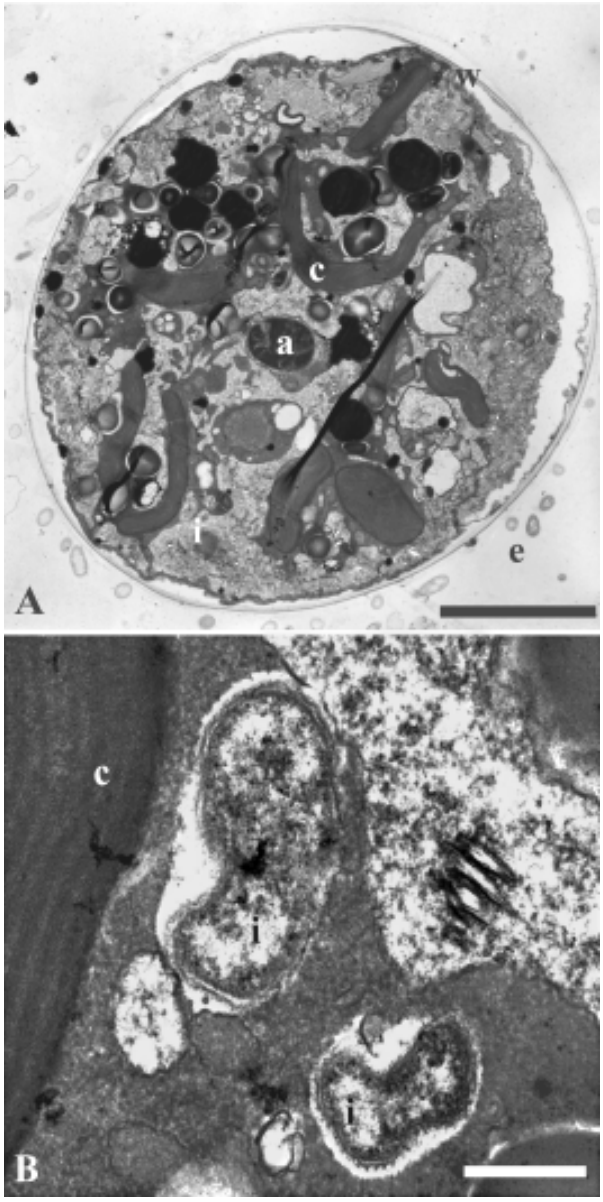

Fig. 2. Mature hypnocysts of *A. tamarense*. A - transmission electron microscopical (TEM) micrograph of a section through an early hypnocyst stage of *A. tamarense*. B - bacteria present in early hypnocysts. Abbreviations: a - accumulation body, c - chloroplasts, e - extracellular bacterium, i - intracellular bacterium, w - cell wall. Scale bars: A - 10μm; B - 1μm.

by the supposedly sporopollenin layer from an electron-permeable layer or gap. Single filaments are not adhesive to each other but are adhesive to different particles in the environment, such as bacteria, diatoms and sand grains. The filaments do not look like mucilage and adhesion does not seem to be mediated by a glue or lectins, although exact composition of the filaments remains unclear. Lectins bind to complementary sugar residues, but observations that hypnocysts also bind to sand grains, silica frustules and plastic Petri dishes contradicts the assumption that adhesion in hypnocysts is lectin-mediated. The filaments have probably the same electrical charge, repulsing each other to avoid agglutination. The adhesion of filaments to particles is probably mediated by a different electrical charge of the particles and the filaments. These layers of adhesive filaments are invaded by some extracellular bacteria but not by other particles. This kind of covering is probably shed off in conventional preparation procedures for scanning electron microscopy and was therefore overlooked in earlier investigations. Gao et al. (1989) described fibrils attached to the outside of the cyst wall of *Scrippsiella* sp., differing in structure from the filaments described here. The former filaments were mentioned to be the starting points for calcification, which does not occur in *Alexandrium tamarense.* The covering of hypnocysts with filaments is probably more common in the genus *Alexandrium* than in other dinophlagellate genera. For example, cysts of *A. catenella* are covered with a mucilaginous substance which appears similar to our filaments in scanning electron micrographs (Meksumpun et al., 1994), but the similarities will have to be confirmed by further transmission electron microscopical investigations. Presence of intracellular bacteria in cysts of the toxic *Alexandrium tamarense* has not been demonstrated so far and introduces a new aspect into the discussion about dinflagellate-bacteria interactions. Before the present investigation, intracellular bacteria have been unequivocally demonstrated only for vegetative stages of toxic dinoflagellates. It remains unclear whether the bacteria associated with dinoflagellates or the dinoflagellate cells alone are able to produces these toxins. The presence of intracytoplasmic bacteria in hypnocysts of *A. tamarense* displays the general potential of toxic bacteria to survive a period of encystment in *A. tamarense.* Our results also show the potential of *A. tamarense* and their intracellular bacteria to form a long-term association (probably for several years) without an obligatory new formation of the endocytobiosis after each dormancy period.

Acknowledgements

I thank P. Hanke-Sautter, I. Polle (both Univ. of Stuttgart, Germany) and H. Halliger (BAH/AWI List/Sylt, Germany) for technical assistance, Dr. M. Elbrächter for hospitality in his laboratory at the BAH/AWI -Wattenmeerstation- List/Sylt, the crew of the research vessel RV HEINCKE for support on board,

all participants of the cruise HE-105 for discussions and finally the BMBF (TEPS-Project: 03F0161, PABS) for funding.

References

Anderson D.M. and Wall D. 1978. The potential importance of benthic cysts of *Gonyaulax tamarensis* and *Gonyaulax excavata* in initiating toxic dinoflagellate blooms. J. Phycol. 14, 224-234.

Anderson D.M., Aubrey D.G., Tyler D.G. and Coats D.W. 1982. Vertical and horizontal distributions of dinoflagellate cysts in the sediments. Limnol. Oceanography. 27, 757-765.

Anderson D.M., Chisholm S.W. and Watras C.J. 1983. The importance of life cycle events in the population dynamics of *Gonyaulax tamaresis*. Mar. Biol. 76, 179-190.

Anderson D.M., Fukuyo Y. and Matsuoka K. 1996. Cyst Methodologies. In: Manual of Harmful Marine Microalgae (Eds. Hallegraeff G.M., Anderson D.M., and Cembella A.E.) UNESCO (Paris) pp. 229-249.

Arzul G., Erard-Le Denn E., Belin C and Nézan E. 1995. Ichthyotoxic events associated with *Gymnodinium cf. nagasakiense* on the Atlantic coast of France. Harmful Algae News 12/13, 8-9.

Biegala I.C., Kennaway G., Alverca E., Lennon J.-F., Vaulot D. and Simon N. 2002. Identification of bacteria associated with dinoflagellates (Dinophyceae) *Alexandrium* spp. using tyramide signal amplification-fluorescent *in situ* hybridization and confocal microscopy. J. Phycol. 38. 404-411.

Brooks J. 1971. Some chemical and geochemical studies on sporopollenin. In: Sporopollenin (Eds. Brooks J., Grant P.R., Muir M., van Gijzel P., and Shaw G.). Academic Press, London, New York. pp. 351-407.

Delgado M., Garces E., Vila M. and Camp J. 1997. Morphological variability in three populations of the dinoflagellate *Alexandrium taylori*. J. Plankton Res. 19, 749-775.

Franca S. 1991. Dinoflagellate toxicity: harmful effects, warning meashures and research trends in Portugal. In: Proceedings of Symposium on Marine biotoxins. (Eds. Fremy J.M. and Maisons-Alfort C.N.E.V.A.). pp. 229-233.

Fritz L., Anderson D.M. and Triemer R.E. 1989. Ultrastructural aspects of sexual reproduction in the red tide dinoflagellate *Gonyaulax tamarensis*. J. Phycol. 25, 95-107.

Fukuyo Y., Takano H., Chihara M. and Matsuoka K. 1990. Red tide organisms in Japan - An illustrated guide. Uchida Rokakuho, Tokyo, pp. 430

Gallacher S., Flynn K.J., Franco J.M., Brueggemann E.E. and Hines H.B. 1997. Evidence for production of paralytic shellfish toxins by bacteria associated with *Alexandrium* spp. (Dinophyta) in culture. Appl. Environ. Microbiol., 63, 239-245.

Gao X., Dodge D.J. and Lewis J. 1989. An ultrastructural study of planozygotes and encystment of a marine dinoflagellate *Scrippsiella* sp. Brit. Phycol. J. 24, 153-165.

Gerdts G., Hummert C., Donner G., Luckas B. and Schutt C. 2002. A fast fluorimetric assay (FFA) for the detection of saxitoxin in natural phytoplankton samples. Mar. Ecol. Prog. Ser.

Kodama M., Ogata T. and Sato S. 1988. Bacterial production of saxitoxin. Agric. Biol. Chem. 52, 1075-1077.

Kodama M., Ogata T., Sakamoto S., Honda T. and Miwanati T. 1990. Production of paralytic shellfish toxins by a bacterium *Moraxella* sp. isolated from *Protogonyaulax tamarensis*. Toxicon. 28, 707-714.

Kopp M., Doucette G.J., Kodama M., Gerdts G. and Schütt C., 1997. Phylogenetic analysis of selected toxic and non-toxic bacterial strains isolated from the toxic dinoflagellate *Alexandrium tamarense*. FEMS Microbiol. Ecol. 24, 251-257.

Lafay B., Rumy R., Rausch de Traubenberg C., Breitmayer V., Gauthier M.J. and Christen R. 1995. *Roseobacter algicola* sp. nov., a new marine bacterium isolated from the phycosphere of the toxin-producing dinoflagellate *Prorocentrum lima*. Int. J. Syst. Bacteriol. 45, 290-296

Lewis J., Kennaway G., Franca S. and Alverca E. 2001. Bacterium-dinoflagellate interactions: investigative microscopy of *Alexandrium* spp. (Gonyaulacales, Dinophyceae). Phycologia. 40, 280-285.

Lu Y.-H., Chai T. and Hwang D.-F. 2000. Isolation of bacteria from toxic dinoflagellate *Alexandrium minutum* and their effects on algae toxicity. J. Nat. Toxins. 9, 409-417.

Medlin L.K., Lange M., Wellbrock U., Donner G., Elbrächter M., Hummert C. and Luckas B. 1998. Sequence comparisons link toxic European isolates of *Alexandrium tamarense* from the Orkney Islands to toxic North American stocks. Europ. J. Protistol. 34, 329-335.

Meksumpun S., Montani S. and Uematsu M. 1994. Elemental components of cyst walls of three marine phytoflagellates, *Chattonella antiqua* (Raphidiophyceae), *Alexandrium catenella* and *Scrippsiella trochoidea* (Dinophyceae). Phycologia. 33, 275-280.

Oshima Y., Bolch C.J., Hallegraeff G.M. 1992. Toxin composition of resting cysts of *Alexandrium tamarense* (Dinophyceae). Toxicon. 30, 1539-1544.

Penna N., Rinaldo A., Montanari D., Di Paolo A. and Penna A. 1993. Mucilaginous masses in the Adriatic Sea in the summer of 1989. Water Res. 27, 1767-1771.

Prokic I., Brümmer F., Brigge T., Görtz H.-D., Gerdts G., Schütt C., Elbrächter M. and Müller W.E.G. 1988. Bacteria of the genus *Roseobacter* associated with the toxic dinoflagellate *Prorocentrum lima*. Protist. 149, 347-357.

Reize I.B. and Melkonian M. 1989. A new way to investigate living flagellated/ciliated cells in agarose. Bot. Acta. 102, 145-151.

Reynolds E. 1963. The use of Lead citrate at high pH as an electron-opaque stain in electron microscopy. 17, 208-212.

Rosenberg R., Lindahl O. and Blanck H. 1988. Silent spring in the Sea. Oceanology 17, 289-290.

Silva E.S.E. 1982. Relationship between dinoflagellates and intracellular bacteria. In: Marine Algae in Pharmaceutical science. Vol. 2 (Eds. Hoppe H.A. and Levring T.). pp. 269-288.

Silva E.S.E. 1990. Intracellular bacteria: the origin of dinoflagellate toxicity. J. Environ. Pathol. Toxicol. and Oncol. 10, 124-128.

Spurr A.R. 1969. A low viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. 26, 31-43.

Taylor J.F.R. 1968. Parasitism of the toxin-producing dinoflagellate *Gonyaulax catenella* by the endoparasitic dinoflagellate *Amoebophyra ceratii*. J. Fisheries Res. Bd. Canada. 25, 2241-2245.

Thomas C.R. 1997. Identifying marine Phytoplankton. Academic Press, Harcourt Brace and Company, San Diego, New York, Boston, London, Sydney, Tokyo, Toronto.

Turpin D.H., Dobell P.E.R. and Taylor F.J.R. 1978. Sexuality and cyst formation in pacific strains of the toxic dinoflagellate *Gonyaulax tamarensis*. J. Phycol. 14, 235-238.

von Stosch H.A. 1965. Sexualität bei *Ceratium cornutum* (Dinophyta). Naturwissenschaften. 52, 112-113.

von Stosch H.A. 1973. Observations on the vegetative reproduction and sexual life cycles of two freshwater dinoflagellates, *Gymnodinium pseudopalustre* and *Woloszynskia apiculata* sp. nov. Br. Phycol. J. 8, 105-134.

Wall D. 1975. Taxonomy of cysts of red tide dinoflagellates. In: Toxic dinoflagellate blooms. (Ed. LoCicero L.). Proceedings of the First International Conference Massachusettes Science and Technology Foundation Wakefield. pp. 249-255.

Address for correspondence: Michael Schweikert. Department of Zoology, Biological Institute, University of Stuttgart, Pfaffenwaldring 57, 70569 Stuttgart, Germany. E-mail: Michael.Schweikert@po.uni-stuttgart.de

Editorial responsibility: Sergei Fokin