Oscillatoria simplicissima: A taxonomical study

A Venter*, A Jordaan and AJH Pieterse
School of Environmental Sciences and Development: Botany, PU for CHE, Potchefstroom 2520, South Africa

Abstract

Oscillatoria simplicissima is a filamentous blue-green alga that was identified in the Vaal River in South Africa. The magnitude of its blooms causes a decline in water quality as well as problems in extracting and purifying water. This species was classified as *Oscillatoria simplicissima* under the order Nostocales and the family Oscillatoriaceae due to its simple unbranched trichome without heterocysts and akinetes. However, it was reclassified under the species *Microcoleus lyngbyaceaus* because of the thick outer walls of its terminal cells. The classification was again changed to *Phormidium simplicissimum.* Therefore, apart from looking at the ultrastructure of this organism this investigation also tried to unravel the classification of this species.

Introduction

Blue-green algae represent only a small proportion of all algal groups in the Vaal River, but they are probably one of the most important, taking into account their potential to be problematic (whether toxin-producing, filter-clogging, scum-forming or discouraging recreational activities). A study done on the development of phytoplankton assemblages in the middle Vaal River (1992 to 1997) showed that *Oscillatoria simplicissima* Gomont, a filamentous blue-green alga, can probably be regarded as the most important bloom-forming blue-green algal species in the Vaal River (Janse van Vuuren, 2001).

Blooms of *O. simplicissima* result in the production of unpleasant odours and tastes in treated water and a general decline of the water quality. Although this blue-green alga is presumably non-toxic in small concentrations, the magnitude of its blooms causes many logistical problems in extracting and purifying water from the Vaal River, leading to increases in purification costs and the loss of large volumes of water.

O. simplicissima was first identified in the Vaal River during 1984 (Pieterse and Steynberg, 1993). Authors such as Geitler (1932) and Desikachary (1959) classified *O. simplicissima* under the order Nostocales and the family Oscillatoriaceae due to its simple unbranched trichome without any heterocysts. Drouet (1968) classified *O. simplicissima* under the species *Microcoleus lyngbyaceaus*, grouping organisms of the Oscillatoriaceae that form terminal cells with thick outer walls, together. Anagnostidis and Komárek (1988) introduced other non-traditional features such as the type of cell division, occurrence of aerotopes (groups of gas vesicles), motility and type of trichome disintegration to classify this organism under the order Oscillatoriales, family Phormidiaceae, subfamily Phormidioideae, species *Phormidium simplicissimum.*

The aims of this investigation were to look at the ultrastructure of *O. simplicissima* as part of a study to understand the growth and physiology of this blue-green alga, but also to investigate the different classification systems of *O. simplicissima*. Because of the uncertainty of the correct classification of the organism studied, it will be called *O. simplicissima* for the purpose of this paper.

* To whom all correspondence should be addressed.
☎ 018 299 2517; fax 018 299 2503; e-mail: plbaav@puknet.puk.ac.za
Received 11 April 2002; accepted in revised form 3 September 2002.

Materials and methods

O. simplicissima batch cultures were grown in a 50% GBG 11 medium (Krüger, 1978) in 250 mℓ Erlenmeyer flasks, at 28°C and at a light intensity of 15 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Filtered filaments were placed on an agar block (5 x 5 x 5 mm) and prepared for investigations using the transmission electron microscope (Phillips CM10).

For the scanning electron microscope (Phillips XL30 Dxi/4), samples (0.5 to 2.0 mℓ) were filtered through an 0.5 µm mesh sized Millipore filter.

Preparations for the transmission electron microscope (TEM)

Agar samples were fixated for 2 to 6 h in Todd's solution (Todd, 1986), washed three times for 10 min in cacodylate buffer. Samples were post-fixated in 0.5% OsO_4 for 1 h and washed 3 times for 10 min in distilled water. Samples were dehydrated through 50%, 70%, 90%, 100% and again 100% acetone for 15 min each. Infiltration with resin was initiated with a 3 h soak in a 1:1 mixture of acetone and resin and left for 3 h. Samples were placed in 100% resin for 5 h and placed in fresh 100% resin for another 2 h. The samples were then embedded in 100 % resin by keeping it for 8 h at 70°C. Thin sections were cut with an ultramicrotome and were retrieved on a copper specimen grid and post-stained with 0.5% uranyl acetate and 0.4% lead citrate.

Preparations for the scanning electron microscope

The filter papers containing the algal filaments were dehydrated in an ethanol concentration series of 10%, 20%, 40%, 60%, 80%, 90%, 95% and twice in 100%. After dehydration, critical-point drying was done with an EM SCOPE TB500 coater, and the material was coated with carbon and gold palladium for 3 min.

Results and discussion

This investigation found that *O. simplicissima* has typical characteristics as described by Geitler (1932), Desikachary (1959) and Anagnostidis and Komárek (1988). *O. simplicissima* is a filamentous alga with uniserially arranged cells that are not constricted at the cross walls (Fig. 1). The straight, unbranched, trichome is dark blue-green, covered with a thin hyaline sheath and is not attenuated or capitated at the apice. Terminal cells are

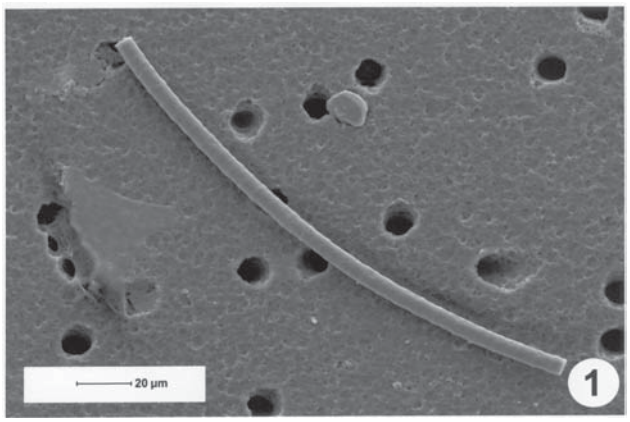

Figure 1
Scanning electron micrograph of a hormogonium of Oscillatoria simplicissima

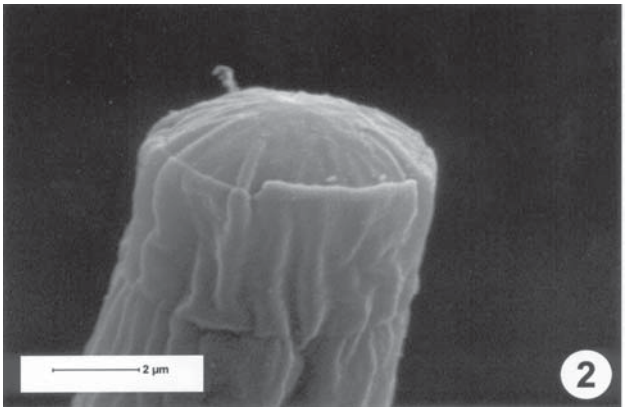

Figure 2
Scanning electron micrograph of the terminal cell of a filament of Oscillatoria simplicissima

hemispherical with a slightly thickened membrane on the outer cell envelope (Fig. 2). Specialised cells, such as akinetes and heterocysts, are lacking. *O. simplicissima* reproduces by forming of short pieces of trichomes, called hormogonia (Fig. 1). Cell division and growth occur over the entire length of the hormogonium to form a mature trichome (Fig. 3). New cross walls (CW) are formed perpendicularly to the long axis of the trichome.

Geitler (1932), Desikachary (1959) and Drouet (1968) found that the cells of *O. simplicissima* are shorter than broad. Desikachary (1959), Drouet (1968) and Anagnostidis and Komárek (1988) included organisms with a width of 8 to 9 µm, 1.5 to 8 µm and 1 to 12 µm respectively. This investigation found that the width of *O. simplicissima* filaments varies between 8 to 12 µm.

According to Anagnostidis and Komárek (1988), Gomont used unstable features, such as the presence of sheaths, to define genera of the Oscillatoriales. These authors stated that the ability of *O. simplicissima* to form sheaths around the trichome depends on environmental conditions. If sheaths are formed they are firm, not lamellated, and adhere to the trichome (Anagnostidis and Komárek, 1988). Desikachary (1959) also used sheath formation to distinguish between *Oscillatoria* species that lack a sheath or those that form a sheath around a single trichome. A thin sheath surrounding the trichome of *O. simplicissima* was observed in this investigation (S; Fig. 4).

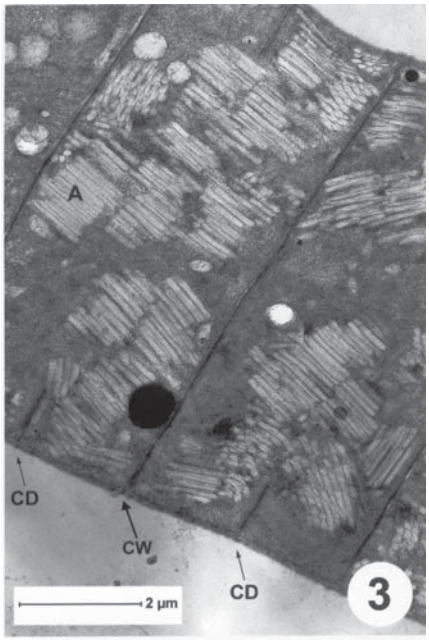

Figure 3
Transmission electron micrograph of a longitudinal section of an Oscillatoria simplicissima *filament*
A = aerotopes, CD = cell division, CW = cross wall

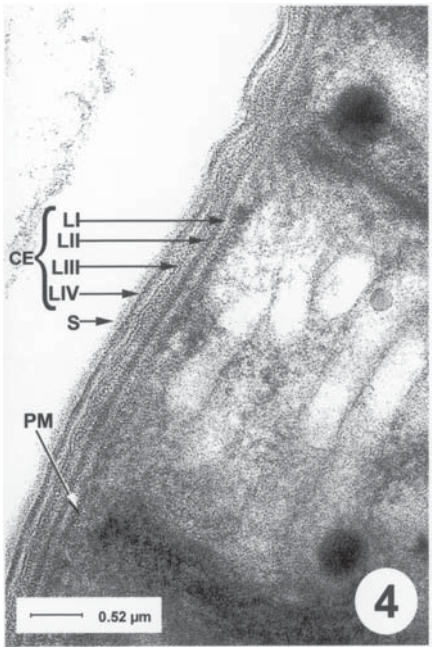

Figure 4
Transmission electron micrograph of the cell envelope of a filament of Oscillatoria simplicissima
CE = cell envelope, LI, LII, LIII, LIV = layers of cell envelope, PM = plasma membrane, S = sheath

The cell envelope (CE) of *O. simplicissima* appears as a multilayered structure under the electron microscope (Fig. 4). Häder and Hoiczyk (1992) used the term cell envelope to describe all the cell layers outside the cytoplasmic membrane. Jost (1965) numbered the layers from I to IV. Layers I and III are electron-transparent, Layer II and IV are electron-dense with a globular

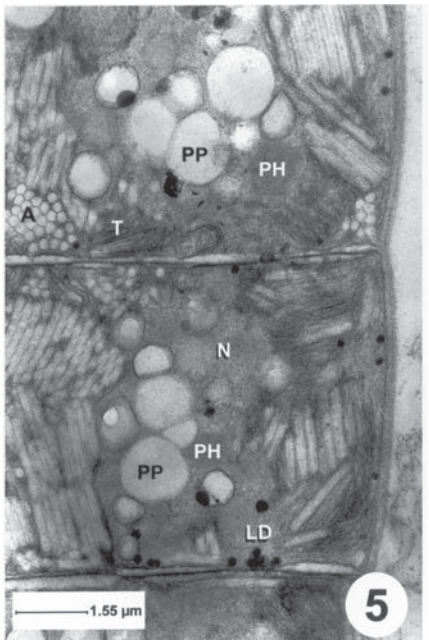

Figure 5
Transmission electron micrograph of a cross-section of a filament of Oscillatoria simplicissima
A = aerotopes, LD = lipid droplet, PH = polyhedral bodies, PP = polyphosphate bodies, N = nucleoplasmic region with DNA and ribosomes, T= thylakoids

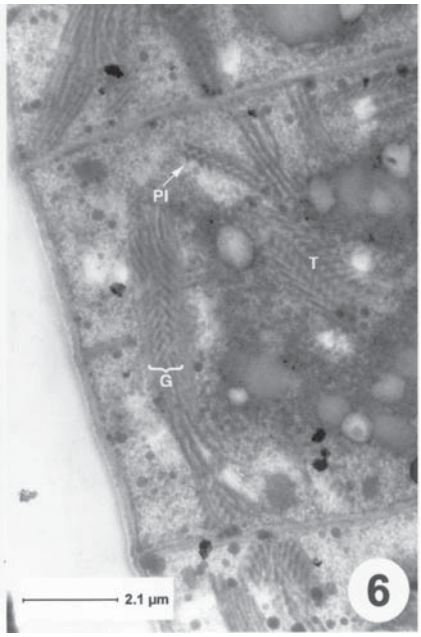

Figure 6
Transmission electron micrograph of the longitudinal section of a filament of Oscillatoria simplicissima *grown on agar for 2 months*
PI = phycobilisomes, G = grana-like stacked arrays of thylakoids (T)

substructure (Fig. 4). However, according to Golecki (1977) and Häder and Hoiczyk (1992) Layer I seems to be an artifact of fixation or embedding, since it is not present in freeze-fracture or cyro-ultrathin sections. Drews (1973) suggested that Layer I is a space between the cell envelope and the cytoplasmic membrane, enriched with enzymes that are active in polymerization of cell envelope precursors. Therefore, Layer II is the innermost layer of the cell envelope (Häder and Hoiczyk, 1992). Drews (1973) found that Layers II and III consist of a murien protein complex but Häder and Hoiczyk (1992) suggested that Layer III could also be an artifact. Layer IV is the outer layer and has a membrane-like structure.

The plasma membrane (PM) of *O. simplicissima* can be seen beneath the cell envelope (Fig. 4). According to Fogg et al. (1973) it is a typical unit membrane about 7 nm thick and acts as a selective, semi-permeable membrane.

The photosynthetic membrane system of *O. simplicissima* consists of flattened vesicles with unit membranes called thylakoids (T; Fig. 5). Grana-like stacked arrays of tightly packed thylakoids with phycobilisomes (PI) were found in filaments of *O. simplicissima* grown on agar (0.5 g agar per 100 mℓ GBG 11 medium and incubated at 28°C and 8 µmol $m^{-2} \cdot s^{-1}$) for two months (Fig. 6). Pankratz and Bowen (1963) also observed grana-like thylakoids in aged cultures of *Symploca muscorum*. Chlorophyll-*a* and phycobilisomes (PI) are light harvesting protein complexes (Lamont and Anderson, 1983) found in Cyanophyta.

Cytoplasmic inclusions present in *O. simplicissima* were identified by shape and are polyglucan granules, lipid droplets (LD; Fig. 5), cyanophycin granules and polyphosphate bodies (PP; Fig. 5). Excess nutrients are stored as insoluble polymers localised in inclusion bodies. The accumulation of excess nutrients may be of critical importance in sustaining blue-green algae in a variety of fresh water habitats when the exogenous sources of nutrients, such as nitrogen and phosphorus, appear to be limiting (Stewart et al., 1978). Polyhedral bodies (PH; Fig. 5) were observed in *O. simplicissima* cells in the nucleoplasmic region. According to Miziorko and Lorimer (1983) these bodies consist of the enzyme ribulose 1,5-biphosphate carboxylase-oxygenase or Rubisco.

Aerotopes (A; Figs. 3 and 5) are conspicuous beehive-like structures observed in the filaments of *O. simplicissima*. Aerotopes were absent in filaments growing for two months on agar (Fig. 6). Gas vesicles in aerotopes are bright, refractive bodies that consist of cylindrical, gas-filled cavities lined by a protein membrane. The proteins are arranged in 4.5 nm wide ribs running at right angles to the cylindrical axis (Lang, 1968). The membrane is rigid, impermeable to water but permeable to all gases. It has been hypothesised that the inner surface of the membrane is hydrophobic and the outer surface hydrophilic (Shively, 1974). According to Walsby (1977) the collapse of gas vesicles is caused by cell turgor pressure. When the cyanobacterium is grown under a low light intensity the cell turgor pressure is low. When the light intensity increases, the rate of photosynthesis and cell turgor increases, thereby collapsing the weaker gas vesicles. Sufficient gas vesicles can be collapsed to result in the cell losing its buoyancy (Walsby and Reynolds, 1980).

Conclusion

Typical features of *O. simplicissima*, as described by Geitler (1932), Desikachary (1959) and Anagnostidis and Komárek (1988) were observed in this investigation. Anagnostidis and Komárek (1988), however, did not observe any aerotopes in *Pormidium simplicissimum*. Figures 7(a) and 7(b) compare a light microscope photo of an *O. simplicissima* filament grown in batch culture for 6 months (Fig. 7(a)), with a filament of *O. simplicissima* grown in batch culture for more than 5 years (Fig. 7(b)). It is clear that *O. simplicissima* grown in culture for a long time does not have aerotopes. Geitler (1932) and Desikachary (1959) both observed

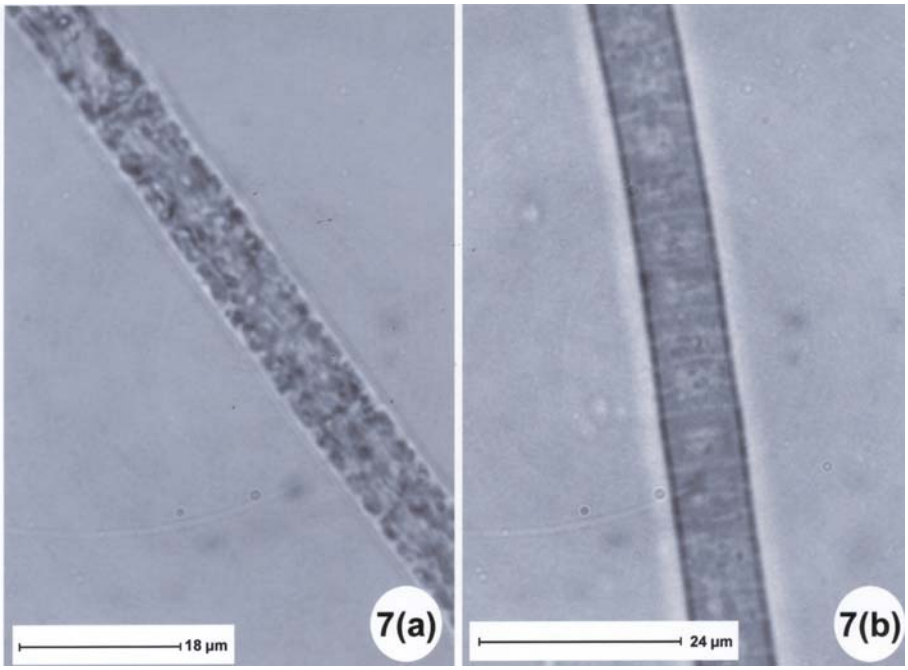

Figure 7(a)
Light microscope photo of a filament of Oscillatoria simplicissima *growing in a batch culture for 6 months, showing the presence of aerotopes*

Figure 7(b)
Light microscope photo of a filament of Oscillatoria simplicissima *growing in culture for 6 years, without any aerotopes*

aerotopes in *O. simplicissima*.

Anagnostidis and Komárek (1988) also stated that the thallus of *Pormidium simplicissimum* occurs in mats but Venter (2000) found that the trichomes of *O. simplicissima* only clumped together when grown under stress conditions such as high temperatures and light intensities. Desikachary (1959) also mentioned that *Oscillatoria* species do not form bundles.

Because of these discrepancies in the classification of Anagnostidis and Komárek (1988) it was decided to use the classification of Geitler (1932) and Desikachary (1959) to classify this organism under the order Nostocales, the family Oscillatoriaceae and the species *Oscillatoria simplicissima*.

References

ANAGNOSTIDIS K and KOMAREK J (1988) Modern Approach to the Classification System of Cyanophytes 3 – Oscillatoriales. *Algol. Stud.* **50-53** 327-472.

DESIKACHARY TV (1959) *Cyanophyta*. Indian Council of Agricultural Research. New Delhi. 686 pp.

DREWS G (1973) Fine Structure and Chemical Composition of the Cell Envelopes. In: Carr NG and Whitton BA (eds.) *The Biology of Blue-Green Algae*. Univ. of California Press, Los Angeles. 676 pp.

DROUET F (1968) *Classification of the Oscillatoriaceae*. The Academy of Natural Sciences of Philadelphia. Pennsylvania. 370 pp.

FOGG GE, STEWART WDP, FAY P and WALSBY AE (1973) *The Cyanobacteria*. Academic Press London. 459 pp.

GEITLER L (1932) Kryptogamen – Flora von Deutschland, Österreich und der Schweiz. *Bnd. 14. Cyanophyceae von Europa*. Akademische Verlagsgesellshaft m.b.H., Leipzig. 1196 pp.

GOLECKI JR (1977) Studies on Ultrastructure and Composition of Cell Walls of the Cyanobacterium *Anacystis nidulans*. *Arch. Microbiol.* **114** 35-41.

HÄDER D and HOICZYK E (1992) Gliding motility. In: Melkonian M (ed.) *Algal Cell Motility*. Chapman and Hall New York. 236 pp.

JANSE VAN VUUREN S (2001) Environmental Variables and the Development of Phytoplankton Assemblages in the Vaal River Between the Rand Water Barrage and Balkfontein. Ph.D. Thesis, PU for CHE, Potchefstroom. 370 pp.

JOST M (1965) Die Ultrastruktur von *Oscillatoria rubescens*. *Arch. Microbiol.* **50** 211-245.

KRÜGER GHJ (1978) The Effect of Physio-Chemical Factors on the Growth Relevant to the Mass Culture of *Microcystis* under Sterile Conditions. Ph.D. Thesis, Univ. of the Orange Free State, Bloemfontein. 134 pp.

LAMONT K and ANDERSON M (1983) Regulation of *Nostoc* sp. Phycobilisome Structure by Light and Temperature. *J. Bacteriol*. **155** (3) 1407-1416.

LANG NJ (1968) The Fine Structure of Blue-Green Algae. *Ann. Rev. Microbiol.* **22** 15-44.

MIZIORKO HM and LORIMER GH (1983) Ribulose-1,5-Bisphosphate Carboxylase-Oxygenase. *Ann. Rev. Biochem.* **52** 507-535.

PANKRATZ HS and BOWEN CC (1963) Cytology of Blue-Green Algae. I. The Cells of *Symploca muscorum*. *Am. J. Bot.* **50** 387-399.

PIETERSE AJH and STEYNBERG MC (1993) Water Quality and Algal Growth in the Loch Vaal. Report on a Consultation Project for Rand Water, Department of Botany and Genetics, UOFS. Rand Water, Vereeniging. 19 pp.

SHIVELY JM (1974) Inclusion Bodies of Prokaryotes. In: Starr MP, Ingraham JL and Raffel S (eds.) *Annual Review of Microbiol.* **28** 167-187. Annual Reviews Inc. California. 512 pp.

STEWART WDP, PEMBLE M and AL-UGAILY L (1978) Nitrogen and phosphorus dtorage and utilization in blue-green algae. *Mitt. Internat. Verein. Limnol.* **21** 224-247.

TODD WJ (1986) Effects of specimen preparation on the apparent ultrastucture of micro-organisms. In: Aldrich HC and Todd WJ (eds.) *Ultrastructure Techniques for Micro-Organisms*. New York: Plenum Press, 87 pp.

VENTER A (2000) The Physiological Characteristics of *Oscillatoria simplicissima*. Ph.D. Thesis, PU for CHE, Potchefstroom. 140 pp.

WALSBY AE (1977) The gas vacuoles of blue-green algae. *Sci. Am.* **237** (2) 90-97.

WALSBY AE and REYNOLDS CS (1980) Sinking and floating. In: Morris I (ed.) *The Physiological Ecology of Phytoplankton*. Blackwell Scientific Publications, Oxford. 625 pp.