RESEARCH LETTER

Description of new filamentous toxic *Cyanobacteria* (*Oscillatoriales*) colonizing the sulfidic periphyton mat in marine mangroves

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Abstract

In this multidisciplinary study, we combined morphological, physiological, and phylogenetic approaches to identify three dominant water bloom-forming *Cyanobacteria* in a tropical marine mangrove in Guadeloupe (French West Indies). Phylogenetic analysis based on 16S rRNA gene sequences place these marine *Cyanobacteria* in the genera *Oscillatoria* (*Oscillatoria* sp. clone gwada, strain *OG*) or *Planktothricoides* ('*Candidatus* Planktothricoides niger' strain *OB* and '*Candidatus* Planktothricoides rosea' strain *OP*; both provisionally novel species within the genus Planktothricoides). Bioassays showed that '*Candidatus* Planktothricoides niger' and '*Candidatus* Planktothricoides rosea' are toxin-producing organisms. This is the first report of the characterization of *Cyanobacteria* colonizing periphyton mats of a tropical marine mangrove. We describe two novel benthic marine species and provide new insight into *Oscillatoriaceae* and their potential role in marine sulfide-rich environments such as mangroves.

Introduction

Mangroves are recognized to be among the most productive ecosystems and are characterized by a high turnover of organic matter mediated by microbial processes (Holguin *et al.*, 2001). Studies have typically focused on the microbial communities of the terrestrial environments (i.e. mangrove soils and sediments) (Rigonato *et al.*, 2012, 2013; Varon-Lopez *et al.*, 2014), and little is known regarding the marine microbial communities (Shamina *et al.*, 2014). The periphyton (close to the submerged roots of *Rhizophora mangle*) dominates primary production in marine mangroves. This primarily anaerobic environment (Attri *et al.*, 2011) serves as an important base of the food web and also regulates water column oxygen dynamics (Alongi, 1990). Microbial communities that grow as microbial mats are a widespread phenomenon (Swift & Nicholas, 1987; Moyer *et al.*, 1995; Stal, 1995; Ward *et al.*, 1998; Wu *et al.*, 2010; D'Silva *et al.*, 2012). Marine mats are highly diverse microbial communities, and include autotrophic and heterotrophic microorganisms tightly coupled within a matrix in periphyton (Mihm *et al.*, 1981; Webster & Negri, 2006). In such context, the cyanobacteria, mostly found in natural aquatic ecosystems (Fogg *et al.*, 1973) including shallow hydrothermal vents (Garcia-Pichel, 1998), intertidal areas (Fenchel, 1998), and freshwater environments (Jungblut *et al.*, 2012), respond rapidly to environmental conditions and constitute major component of the microbiota in mangrove ecosystem along tropical and subtropical coasts (Kathiresan & Bingham, 2001). *Cyanobacteria* actively participate in biomineralization of organic matter and biotransformation of minerals in mangrove ecosystems through photosynthesis, nitrogen and carbon fixation, and phosphorus accumulation (Allen, 1984; Feller & Sitnik, 1996). In these sulfide-rich environments (Fike *et al.*,

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2008), mechanisms of adaptation to sulfide have been identified in *Cyanobacteria* to escape sulfide toxicity and to carry out anoxygenic photosynthesis (Cohen *et al.*, 1986).

Over the past three decades, some species of prokaryotes or unicellular eukaryotes have emerged as a threat for human health or for the environment. Unpredictable outbreaks of unicellular blooms such as *Cyanobacteria* and algal taxa associated with toxins, cyanotoxins, and phycotoxins, are becoming more widespread and persistent because of the eutrophic state of enclosed and semi-enclosed coastal waters surrounding numerous countries (Viviani, 1992; Lee *et al.*, 2002). Among these problematic unicellular organisms, cyanobacteria, predominantly obligate oxygenic phototrophs (Schopf, 1970; Schopf & Walter, 1982), are associated with high toxicity. Thus, the assessment of toxic *Cyanobacteria* in marine mangrove is a subject of great interest.

We used combined approaches to identify and characterize filamentous microorganisms colonizing the periphyton of marine mangroves to explore the contribution of *Cyanobacteria* to mangrove microbial communities and to understand the structure of *Cyanobacteria* communities in these marine ecosystems. We also investigated toxin production and identified major phycobilin pigment contents by spectral imaging to further characterize these microogranisms.

Materials and methods

Origin of mat samples

Samples were collected by snorkeling (*c.* 0.5 m depth) and 50-mL syringes were used to recover the mat on the sediment from the periphyton between *R. mangle* roots of the marine mangrove in Guadeloupe (F. W. I.) ('La manche à eau'; W61°35′, N16°15′) (Mantran *et al.*, 2009). Once back in the lab (within 1 h after collection), living samples of each strain were separated from associated meiofauna under a light microscope. The samples were then washed with 0.22 μm filtered seawater prior to use in cellular or molecular experiments as described below.

Culture media and growth conditions

All *Cyanobacteria* cultures were initiated, maintained, and cultivated in BG11 medium (Stanier *et al.*, 1971; Guillard, 1973, 1975) supplemented with 2 mM Na_2S, 5 mM $NaHCO_3$, vitamins (vitamin B12, thiamine HCl, and biotin), and artificial seawater adjusted to 28 practical salinity units(PSU, UNESCO, 1981a, b).

Cyanobacteria strains were grown without agitation in 250-mL flasks (Polystyrene, Iwaki, Asahi Techno Glass, Japan) containing 75 mL liquid medium with an inoculum of 10 mL. The strains were grown at 25 °C with 30 μmol m^{-2} s^{-1} (μE) irradiance (photoperiod 12L : 12D) (measured with a testo 540 lux meter, Testo AG, Lenzkirch, Germany). Growth was checked daily with a SZ 61 microscope (Olympus). Cell counts were performed with a hemocytometer (Malassez counting chamber).

16S rRNA gene sequence analysis

DNA for PCR was extracted from 10 filaments of each species after three heat-shock cycles (−20 °C, + 95 °C, 5 min each) in 20 μL of distilled water. 16S rRNA genes were amplified and sequenced with CYA106F and CYA781R (b) primers (Supporting Information, Data S1). PCR reactions were run in a Thermal Cycler with one cycle of 95 °C for 5 min, then 30 cycles of 95 °C for 1 min, 55 °C for 1 min and 72 °C for 1 min, followed by 72 °C for 7 min. DNA sequencing was carried out by GATC Biotech (http://www.gatc-biotech.com).

Phylogenetic analysis

The 16S rRNA gene sequences were aligned and analyzed by comparison with the BLAST tool of the National Center of Biotechnology information (NCBI) (http://www.ncbi.nlm.nih.gov) database (Altschul *et al.*, 1990). Phylogenetic analyses were performed with GENEIOUS VERSION PRO 5.4.4 software created by biomatters (http://www.geneious.com). Sequences were aligned with MUSCLE and alignments were checked manually. A phylogenetic tree was generated according to the neighbor-joining method (Saitou & Nei, 1987) with Tamura-Nei (Tamura & Kumar, 2002) as a genetic distance model.

Environmental scanning electron microscopy (ESEM)

Cyanobacteria filaments were fixed at 4 °C in 2.5% glutaraldehyde in 0.1 M pH 7.2 cacodylate buffer, which was made isosmotic with seawater by the addition of sodium chloride and calcium chloride. The filaments were rinsed quickly in deionized water to remove the salts immediately prior to observation with an ESEM (Quanta 250, FEI) operating at 12 kV under an environmental pressure of 7 Torrs.

Microscopic spectral imaging

Exponential-phase cells were used to determine phycobiliprotein absorption spectra from single cells *in vivo*. Cell filaments were transferred onto glass slides (SuperFrost

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Ultra Plus, Menzel-Glaser, Braunschweig, Germany) and covered with a cover slip (Menzel-Glaser, Braunschweig, Germany). Absorption spectra of living microorganisms were analyzed at room temperature by linear spectral imaging from 500 to 700 nm, which allows the identification of major phycobiliproteins with a confocal laser microscope (TCS SP5 II AOBS; Leica, Wetzlar, Germany) through a 68× objective lens. Individual pigments were identified based on their absorption properties.

Toxicity biotests with *Artemia salina*

Dried cysts of the crustacean *Artemia salina* (Brine shrimp) were purchased from Dohse Aquaristik KG (Grafschaft-Gelsdorf, Germany). Eggs (*c.* 1.6×10^5 cysts g^{-1}) were hatched in artificial seawater at *c.* 25 °C with salinity adjusted to 28 PSU, with vigorous bubbling from the bottom of the tank to give a high oxygen concentration and with illumination at a constant surface light intensity of 2000 lux. After 24 h, hatched *A. salina* cysts, nauplii, exhibiting phototropic movements, were collected with a light source and Pasteur pipette (Campbell *et al.*, 1994). Bioactivity of the samples was monitored according to the brine shrimp lethality test (Guidi-Rontani *et al.*, 2010). Briefly, the toxicity assay was performed in triplicate with 50 μL of larval suspension and about 10–15 nauplii per well (96-multiwell polystyrene plate) at room temperature. *Cyanobacteria* cultures were centrifuged to separate the culture medium and the trichomes. Cellular homogenates were obtained by a tissue grinder (Kontes Glass Company, Vineland, NJ). *Artemia salina* mortality was recorded periodically with a binocular dissecting microscope (SZ 61, Olympus) during 48 h. *Artemia salina* adults were defined as dead when no movements were registered within 60 s. Regression analyses were used for the modeling and analyzing of the results (Cetin *et al.*, 2011). The results of toxicity analyses were tested by analysis of variance (ANOVA).

Results and discussion

Morphological analysis

We collected filamentous microorganisms from extensive white periphyton mats (Fig. 1a) associated with 'pigmented' patches, 'black' mats (Fig. 1b), and 'pink' mats, of the tropical marine mangrove in Guadeloupe. We used light microscopy (bright field) to examine periphyton mats and noticed that the white periphyton mats were composed of a dense benthic filamentous network containing mostly large *Beggiatoa*-like filaments (sulfur-oxidizing bacteria) (Jean, 2013) and few filamentous *Cyanobacteria* (Fig. 1c). In contrast, the microbial community of the 'pigmented' patches was composed mostly of filamentous *Cyanobacteria* (Fig. 1b and d). We noticed a striking difference in filament color including 'black' (designated strain *OB*), 'green' (designated strain *OG*), and 'pink' (designated strain *OP*), reflecting differences in types or amount of pigment (Fig. 1d).

Cell culture enables the study of organism physiology; therefore, the first task to achieve following the isolation of filaments was to define cell culture conditions for these

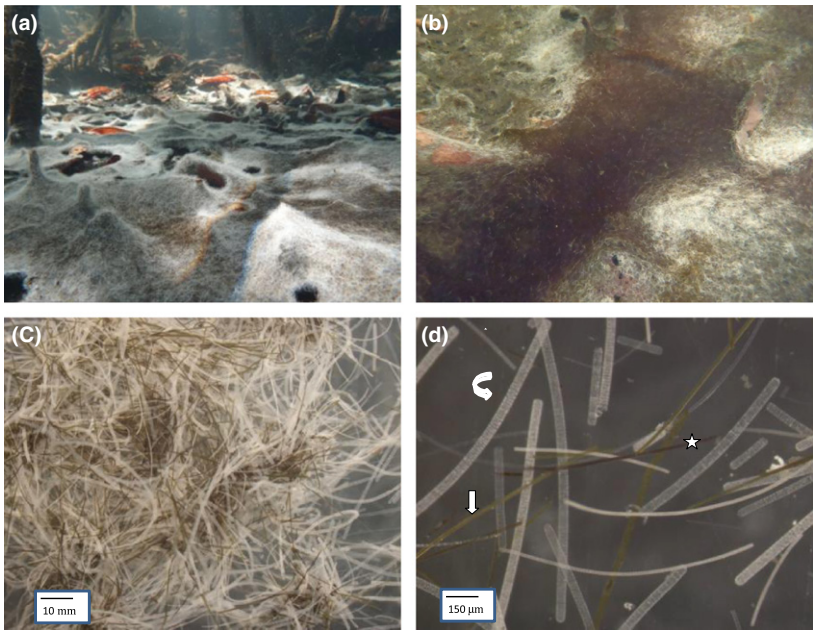

Fig. 1. Underwater pictures of periphyton mats covering sediment and leaves fallen from *Rhizophora mangle* trees in the marine mangrove environment. (a) White periphyton mat mostly composed of sulfur-oxidizing bacterial filaments belonging to the genus *Beggiatoa* sp. The white color of the mat results from the accumulation of elemental sulfur granules inside cells. (b) Black mats mostly composed of benthic cyanobacterial filaments named *OB*. (c) Light micrograph of the white periphyton mat showing white filamentous sulfur-oxidizing bacteria and a few brown benthic cyanobacteria. (d) Higher magnification of the bacterial filaments from the white mat. *Beggiatoa* sp. (large white filaments designated with a white curved arrow) strains are more abundant than green *Cyanobacteria OG* (white arrowhead) and the *OP* strain (white star).

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isolates. As described in 'Materials and methods', *OB* and *OP* isolates were successfully cultivated under similar growth conditions in BG11 medium. In growth conditions, we observed the release of filament fragments (*c.* 5–10 cells) such as hormogonia-like filaments suggesting reproduction by trichome fragmentation. Interestingly, sediment samples displayed motile trichomes resulting in an 'Ashley's knot-like' formation of filaments. Similarly, trichomes exhibited gliding movements on agar medium in response to light (*c.* 50 μE irradiance; data not shown). Therefore, the mat-forming organisms may use gliding motility to reach the chemocline frontier (sunlight at the surface of sediment and oxygen (O_2)/hydrogen sulfide (H_2S) at the interface). We analyzed *Cyanobacteria* growth phases either in aerobic or anaerobic conditions. Interestingly, we observed that *OB* cells grow either aerobically or anaerobically, whereas *OP* requires oxygen (data not shown). This suggests that *OP* isolates do not carry out anoxygenic photosynthesis.

We examined the filaments by light microscopy (bright field). Each filament constituted of a chain of cells (trichomes). Trichomes were solitary, generally straight or slightly waved and never branched: *OP* (Fig. 2a), *OB* (Fig. 2b) and *OG* (Fig. 2c). Trichome length changed considerably as a function of culture conditions (data not shown); therefore, we did not consider this morphological character for taxonomic characterization. We examined the trichomes of these microorganisms with an ESEM; the trichomes of each isolate appeared covered with thin and clear sheaths (mucilage envelopes) (*OP* Fig. 2d, *OB* Fig. 2e and *OG* Fig. 2f). These filaments were similar in shape and possessed disk-shaped cells that were much greater in width than in length (5–10 μm wide and 1.25–2.5 μm long). All analyzed strains had similar shaped terminal cells, which were slightly rounded with no hoodlike part such as calyptra (Fig. 2c). These characteristics of cell morphology and oscillation in the movement pointed to *Cyanobacteria* such as the *Oscillatoriaceae* family section III described by Rippka *et al.* (1979).

Pigment analysis

We performed microscopic spectral imaging of *Oscillatoriaceae* strains (Kühl & Polerecky, 2008) at room temperature to identify the predominant phycobilin pigment composition. This minimally invasive approach

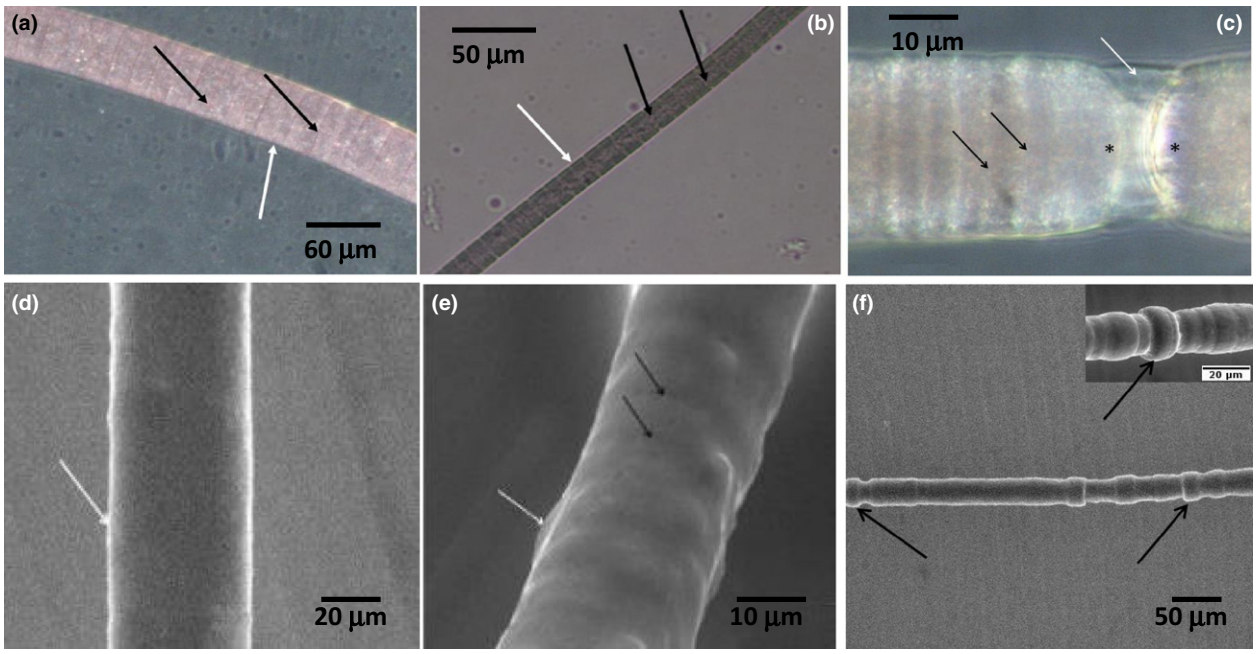

Fig. 2. Light and ultrastructural micrographs of *Oscillatoriaceae* strains. White arrows indicate the sheath; black arrows indicate the cell membranes. (a) Light microphotograph of *OP* solitary trichome characterized by a general pinky color. The filament is made up of a linear colony of disk-shaped *Cyanobacteria* separated from each other by their cell membranes. (b) Light microphotograph of *OB* solitary trichome characterized by a black color. *OP* and *OB* trichomes appear similar in shape. (c) Light microphotograph of *OG* trichome. The asterisk (*) indicates rounded apical cells. (d) Environmental SEM microphotograph of *OP* trichome. The external sheath completely covers all the disk-shaped *Cyanobacteria* composing the trichome, obstructing observation of these bacteria. (e) Environmental SEM microphotograph of *OB* trichome. The sample was dehydrated (observation under a higher vacuum) to enable the visualization of the sheath and separation between adjacent disk-shaped cyanobacteria. (f) Environmental SEM microphotograph of *OG* trichome.

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showed that the *in vivo* absorption spectra of *OB, OG,* and *OP* organisms were similar (Fig. 3). Indeed, the *in vivo* absorption spectrum of each organism possessed two large absorption peaks around 570 and 700 nm. These results indicate that the dominant phycobilin pigments in *OB*, *OG*, and *OP* are similar, with all strains containing both phycoerythrin-like (PE) and phycocyanin-like (PC) dominant pigments. In contrast, these microorganisms differed with regard to the amount of pigment. As expected, the highest peak in *OP* trichomes with a pinkish color corresponded to a phycoerytrin-like pigment and this isolate had a high PE : PC ratio (Fig. 3a), whereas the highest peak in *OG* trichomes with a greenish color corresponded to a phycocyanin-like pigment and therefore this isolate had a low PE : PC ratio (Fig. 3b). Lastly, the abundance of PE and PC was equivalent in *OB* trichomes with a darkish color (Fig. 3c). Interestingly, when *OB* and *OP* strains were grown under red or green light, their trichome color did not change (data not shown). These data suggest that these strains cannot undergo complementary chromatic adaptation (Grossman, 2003). Furthermore, these three distinct spectral signatures strongly suggest that these three benthic phototrophic organisms use different regimens to carry out photosynthesis. Organisms that grow in shallow waters tend to contain phycobilins that can capture yellow/red light; therefore *OB*, *OG*, and *OP* probably exhibit different spatial/temporal dynamics within the periphyton as function of daylight.

Toxicity

In an attempt to explore the potential toxicity of the *OB* and *OP* strains, we evaluated the toxicity of crude cellular extracts against larvae of the brine shrimp *A. salina* (Kiviranta *et al.*, 1991). We compared first the mortality of larvae exposed to the negative control or to supernatant from either culture medium for 12, 24, or 48 h (Fig. 4). We found no significant difference between the control, the *OP* culture medium and the *OB* culture medium in terms of mortality (control and *OP* culture medium*:* $F = 0.00$, 1 d.f.; control and *OB* culture medium: $F = 0.40$, 1 d.f.) (Data S2). We then tested the putative toxicity of the crude cellular extracts. Interestingly, mortality in the presence of the *OP* or *OB* crude cellular extracts were significantly higher than in the presence of the negative control (control and *OP:* $F = 5.32$, 1 d.f.; $P < 0.01$; control and *OB*: $F = 19.82$, 1 d.f., $P < 0.05$). These results reveal that *OB* and *OP* strains are toxic for the early larval stages of *A. salina* (instar I and II larvae) and therefore suggest that these strains produce toxins. These results provide evidence for the production of toxins by marine *Cyanobacteria* strains and are in agreement with the findings of Méjean *et al.* (2010). However, these

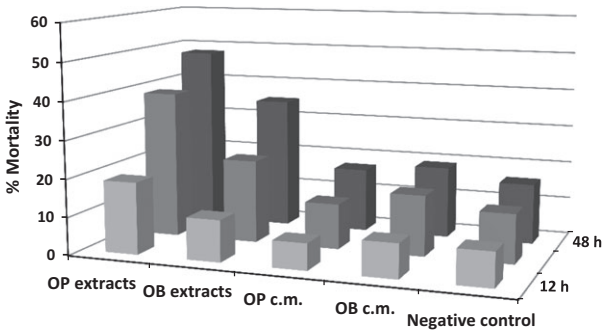

Fig. 4. Toxicity of *Oscillatoriaceae* strains. Mortality of larvae of the brine shrimp *Artemia salina* (%) treated with culture medium (c.m.) or crude cellular extracts of *OB* or *OP*.

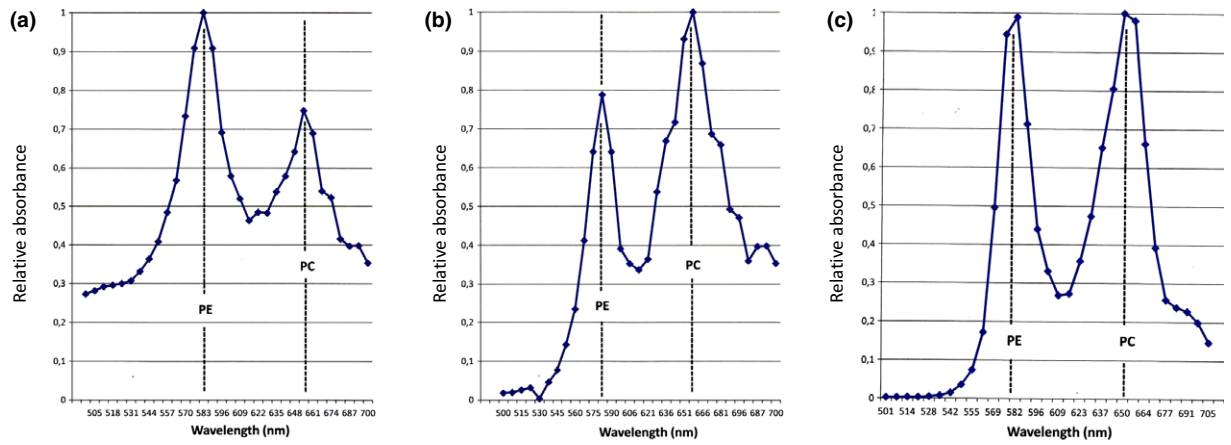

Fig. 3. Normalized *in vivo* absorption spectra for *Oscillatoriaceae* strains. Absorption spectra of major phycobiliproteins analyzed by linear spectral imaging from 500 to 700 nm: (PE) phycoerythrin-like; (PC) phycocyanin-like. (a) *OP* organism. (b) *OG* organism. (c) *OB* organism.

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organisms displayed different levels of toxicity, *OP* showing a more severe toxicity ($F = 6.71$, 1 d.f., $P < 0.05$). The culture medium of *OB* and *OP* strains were not toxic for larvae, suggesting that toxic compounds were not released into the medium. However, toxin secretion in phytoplankton cells may vary widely in relation to the growth phase and external biotic environmental factors, such as salinity, temperature, light intensity, and nutrient availability.

Phylogenetic analysis

We amplified and sequenced the 16S rRNA genes to ascertain the phylogenetic positions of the isolated cyanobacteria. For phylogenetic analysis, we included type species of the genus *Oscillatoria,* such as *Oscillatoria princeps* (Gomont), *O. laetevireus,* and representatives of each group corresponding to the new taxonomic descriptions by Suda *et al.* (2002). Phylogenetic analysis based on partial 16S rRNA gene sequences (628 base pairs in length) revealed a high level of sequence identity between *OB, OP* (> 98%) and group IV cluster water bloom-forming oscillatorioids belonging to the family *Phormidiaceae* (order *Oscillatoriales*). The highest 16S rRNA gene sequence similarity was shared with *Planktothricoides raciborskii* (96%). Thus, we assigned *OB* and *OP* to the genus *Planktothricoides* in reference to the oscillatorioids group IV cluster (Suda *et al.*, 2002) (Fig. 5). Interestingly, *OB* and *OP* could be clearly distinguished by properties such as phycobilin pigment composition, which is an important characteristic in taxonomic identification. Therefore, in reference to phycobilin pigment composition, we propose to name *OB* and *OP*, '*Candidatus* Planktothricoides niger' and '*Candidatus* Planktothricoides rosea', respectively. Group I water bloom-forming oscillatorioids are subdivided into group I-pc and group I-pe according to PC/PE composition; therefore, we also propose to divide group IV into two subgroups, group IV-pc (*P. raciborskii*) and group IV-pc-pe ('*Candidatus* Planktothricoides niger' and '*Candidatus* Planktothricoides rosea') based on this

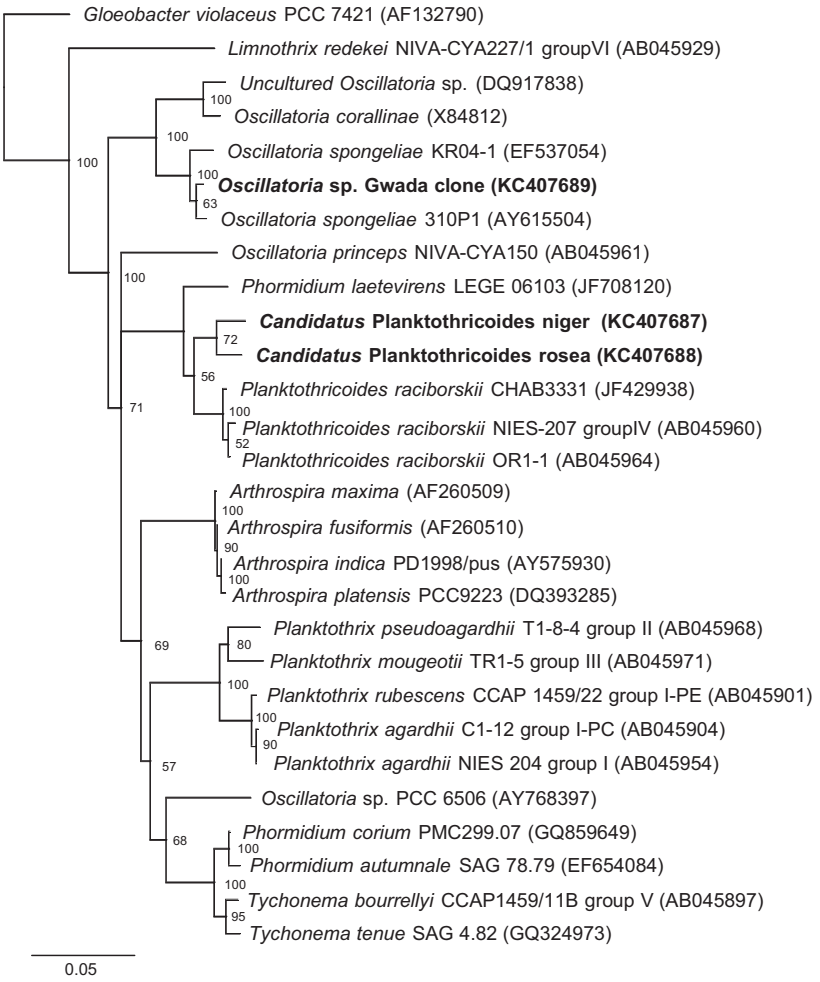

Fig. 5. Phylogenetic analysis based on alignment of 16S rRNA gene sequence (628 bp). Sequences from other species were obtained from GenBank. Node robustness was assessed by performing 1000 bootstrap replications; only bootstrap values above 50% are indicated at the nodes of the tree. *Gloeobacter violaceus* PCC7421 was used as an out-group for the phylogenetic analysis.

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property. Further studies will be needed to determine whether differences in phycobilin pigment content are correlated with the occurrence of these *Planktothricoides* species and may be used as 'signatures'. Therefore, in agreement with the findings of Suda *et al.* (2002) and Komarek & Komarkova (2004), our results suggest that 16S rRNA gene sequence analysis is not enough to classify closely related species. Interestingly, our data suggest that the genera *Planktothricoides* produces toxins.

Phylogenetic analysis identified an *OG* clone as a sister of the *O. spongeliae* clade (Fig. 5), which is a cyanobacterial symbiont of marine sponges (Ridley *et al.*, 2005). The 16S rRNA gene sequence of this clone shares high similarity with that of *O. spongeliae* (99%). We propose to name this *OG* strain *Oscillatoria* sp. clone gwada in reference to the phylum to which they belong, and to the sampling site Guadeloupe (Gwada). The symbiotic relationship between *Cyanobacteria* and their hosts is currently not well defined. Therefore, more studies are needed to characterize the putative symbiotic *Oscillatoria* sp. clone gwada and to determine the eventual complex interactions between this organism and Caribbean mangrove sea sponges.

In addition, future research should explore further the toxic properties of each strain and examine the environmental factors that affect toxin production. These toxin(s) may be identified by HPLC and GC-MS analysis.

Nucleotide sequence accession numbers

Oscillatoria sp. *gwada* clone, '*Candidatus* Planktothricoides niger' and '*Candidatus* Planktothricoides rosea' sequences obtained in this study were deposited in the GenBank database under accession numbers KC407687 to KC407689.

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References

Allen MM (1984) Cyanobacterial cell inclusions. *Annu Rev Microbiol* **38**: 1–25.

Alongi DM (1990) Effect of mangrove detrital outwelling on nutrient regeneration, oxygen fluxes in coastal sediments of the central Great Barrier Reef lagoon. *Estuar Coast Shelf Sci* **31**: 581–598.

Altschul SF, Gish W, Miller W, Myers EW & Lipman DJ (1990) Basic local alignment search tool. *Mol Biol* **215**: 403–410.

Attri K, Kerkar S & LokaBharathi PA (2011) Ambient iron concentration regulates the sulfate reducing activity in the mangrove swamps of Diwar, Goa, India. *Estuar Coast Shelf Sci* **95**: 156–164.

Campbell DL, Lawton LA, Beattie KA & Codd GA (1994) Comparative assessment of the specifity of the brime shrimp and Microtox assays to hepatotoxic (microcystin-LR containing) *Cyanobacteria*. *Environ Toxicol* **9**: 71–77.

Cetin MH, Ozcelik B, Kuram E & Demirbas E (2011) Evaluation of vegetable based cutting fluids with extreme pressure and cutting parameters in turning of AISI 304L by Taguchi method. *J Clean Prod* **19**: 2049–2056.

Cohen Y, Jørgensen BB, Revsbech NP & Poplawski R (1986) Adaptation to hydrogen sulfide of oxygenic and anoxygenic photosynthesis among *Cyanobacteria*. *Appl Environ Microbiol* **51**: 398–407.

D'Silva SM, Anil AC, Naik RK & D'Costa MP (2012) Algal blooms: a perspective from the coasts of India. *Nat Hazards (Dordr)* **63**: 1225–1253.

Feller IC & Sitnik M (1996) *Mangrove Ecology: A Manual for a Field Course*. Smithsonian Institution, Washington, DC.

Fenchel T (1998) Artificial cyanobacterial mats: structure and composition of the biota. *Aquat Microb Ecol* **14**: 241–251.

Fike DA, Gammon CL, Ziebis W & Orphan VJ (2008) Micron-scale mapping of sulfur cycling across the oxycline of a cyanobacterial mat: a paired nanoSIMS and CARD-FISH approach. *ISME J* **2**: 749–759.

Fogg GE, Stewart WDP, Fay P & Walsby AE (1973) *The Blue-Green Algae*. Academic Press, New York, NY.

Garcia-Pichel F (1998) Solar ultraviolet and the evolutionary history of the *Cyanobacteria*. *Orig Life Evol Biosph* **28**: 321–347.

Grossman AR (2003) A molecular understanding of complementary chromatic adaptation. *Photosynth Res* **76**: 207–215.

Guidi-Rontani C, Maheswari U, Jabbari K & Bowler C (2010) Comparative ecophysiology and genomics of the toxic unicellular alga *Fibrocapsa japonica*. *New Phytol* **185**: 446–458.

Guillard RRL (1973) Division rates. *Handbook of Phycological Methods – Culture Methods and Growth Measurements* (Stein JR, Ed), pp. 289–311. Cambrige University Press, Cambridge, UK.

Guillard RRL (1975) Culture of phytoplankton for feeding marine invertebrates. *Culture of Marine Invertebrate Animals* (Smith WL & Chanley MH, eds), pp. 26–60. Plenum Press, New York, NY.

Holguin G, Vazquez P & Bashan Y (2001) The role of sediment microorganisms in the productivity, conservation, and rehabilitation of mangrove ecosystems: an overview. *Biol Fertil Soils* **33**: 265–278.

Jean MNR (2013) Etude de la matte sulfo-oxydante de la mangrove de Guadeloupe: Caractérisation des microorganismes principaux des familles *Beggiatoaceae et*

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Oscillatoriaceae. Diplôme de Doctorat, Université des Antilles et de la Guyane, Pointe-à-Pitre, Guadeloupe, France.

Jungblut AD, Vincent WF & Lovejoy C (2012) Eukaryotes in Arctic and Antarctic cyanobacterial mats. *FEMS Microbiol Ecol* **82**: 416–428.

Kathiresan K & Bingham BL (2001) Biology of mangroves and mangrove ecosystems. *Adv Mar Biol* **40**: 81–251.

Kiviranta J, Sivonen K & Niemela SI (1991) Detection of toxicity of *Cyanobacteria* by *Artemia salina* bioassay. *Environ Toxicol Water Qual* **6**: 423–436.

Komarek J & Komarkova J (2004) Taxonomic review of the cyanoprokaryotic genera *Planktothrix* and *Planktothricoides*. *Czech Phycol Olomouc* **4**: 1–18.

Kühl M & Polerecky L (2008) Functional and structural imaging of phototrophic microbial communities and symbioses. *Aquat Microb Ecol* **53**: 99–118.

Lee CK, Kim HC, Lee SG, Jung CS, Kim HG & Lim WA (2002) Abundance of harmful algae, *Cochlodinium polykrikoides, Gyrodinium impudicum* and *Gymnodinium catenatum* in the coastal area of South Sea OF Korea and their effects of temperature, salinity, irradiance and nutrient on the growth in culture. *J Korean Fish Soc* **34**: 536–544.

Mantran M, Hamparian R & Bouchereau JL (2009) Géomorphologie et hydrologie de la lagune de la Manche-à-Eau (Guadeloupe, Antilles françaises); Geomorphology and hydrology of the Manche-à-Eau lagoon (Guadeloupe, French West Indies). *Géomorphol: Relief Process Environn* **3**: 199–210.

Méjean A, Peyraud-Thomas C, Kerbrat AS, Golubic S, Pauillac S, Chinain M & Laurent D (2010) First identification of the neurotoxin homoanatoxin-a from mats of *Hydrocoleum lyngbyaceum* (marine *Cyanobacterium*) possibly linked to giant clam poisoning in New Caledonia. *Toxicon* **56**: 829–835.

Mihm J, Banta WC & Loeb GI (1981) Effects of adsorbed organic and primary fouling films on bryozoan settlement. *J Exp Mar Biol Ecol* **54**: 167–169.

Moyer CL, Dobbs FC & Karl DM (1995) Phylogenetic diversity of the bacterial community from a microbial mat at an active, hydrothermal vent system, Loihi Seamount, Hawaii. *Appl Environ Microbiol* **61**: 1555–1562.

Nübel U, Garcia-Pichel F & Muyzer G (1997) PCR primers to amplify 16S rRNA genes from *Cyanobacteria*. *Appl Environ Microbiol* **63**: 3327–3332.

Ridley CP, Bergquist PR, Harper MK, Faulkner DJ, Hooper JNA & Haygood MJ (2005) Speciation and biosynthetic variation in four dictyoceratid sponges and their cyanobacterial symbiont, *Oscillatoria spongeliae*. *Chem Biol* **12**: 397–406.

Rigonato J, Alvarenga DO, Andreote FD, Dias ACF, Melo IS, Kent A & Fiore MF (2012) Cyanobacterial diversity in the phyllosphere of a mangrove forest. *FEMS Microbiol Ecol* **80**: 312–322.

Rigonato J, Kent AD, Alvarenga DO, Andreote FD, Beirigo RM, Vidal-Torrado P & Fiore MF (2013) Drivers of cyanobacterial diversity and community composition in mangrove soils in south-east Brazil. *Environ Microbiol* **15**: 1103–1114.

Rippka R, Deruelles J, Waterbury JB, Herdman M & Stanier RS (1979) Generic assignments, strain histories and properties of pure cultures of *Cyanobacteria*. *J Gen Microbiol* **111**: 1–61.

Saitou N & Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**: 406–425.

Schopf JW (1970) Precambrian microorganisms and evolutionary events prior to the origin of vascular plants. *Biol Rev* **45**: 319–352.

Schopf JW & Walter MR (1982) Origin and early evolution of *Cyanobacteria*: the geological evidence. *The Biology of Cyanobacteria*, (Carr NG & Whitton BA, eds), pp. 543–564. Blackwell Scienti®c Publications, Oxford.

Shamina M, Saranya T & Ram AT (2014) Cyanobacterial biodiversity at mangrove vegetation of Kadalundi, Kerala. *J Microbiol* **3**: 15–16.

Stal LJ (1995) Physiological ecology of *Cyanobacteria* in microbial mats and other communities. *New Phytol* **131**: 1–32.

Stanier RY, Kunisawa R, Mandel M & Cohen-Bazire G (1971) Purification and properties of unicellular blue-green algae (order *Chroococcales*). *Bacteriol Rev* **35**: 171–205.

Suda S, Watanabe MM, Otsuka S, Mahakahant A, Yongmanitchai W, Nopartnaraporn N, Liu Y, & Day JG (2002) Taxonomic revision of water bloom–forming species of oscillatorioid *Cyanobacteria*. *Int J Syst Evol Microbiol* **52**: 1577–1595.

Swift DR & Nicholas RB (1987) *Periphyton and Water Quality Relationships in the Everglades Water Conservation Areas 1978–1982*. Technical publication 87-2. South Florida Water Management District. West Palm Beach, FL.

Tamura K & Kumar S (2002) Evolutionary distance estimation under heterogeneous substitution pattern among lineages. *Mol Biol Evol* **19**: 1727–1736.

UNESCO (1981a) Background papers and supporting data on the Practical Salinity Scale, 1978. *Unesco Tech Pap Mar Sci*, **37**: 143.

UNESCO (1981b) Background papers and supporting data on the international equation of state of sea water 1980. *Unesco Tech Pap Mar Sci*, **38**: 192.

Varon-Lopez M, Dias ACF, Fasanella CC, Durrer A, Melo IS, Kuramae EE & Andreote FD (2014) Sulphur-oxidizing and sulphate-reducing communities in Brazilian mangrove sediments. *Environ Microbiol* **16**: 845–855.

Viviani R (1992) Eutrophication, marine biotoxins, human health. *Marine Coastal Eutrophication* (Vollenweider RA, Marchetti R & Viviani R, eds), pp. 631–662. Elsevier Science, Amsterdam, the Netherlands.

Ward DM, Bateson MM, Ferris MJ & Nold SC (1998) A natural view of microbial biodiversity within hot spring

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cyanobacterial mat communities. *Microbiol Mol Biol Rev* **62**: 1353–1370.

Webster NS & Negri AP (2006) Site-specific variation in Antarctic marine biofilms established on artificial surfaces. *Environ Microbiol* **8**: 1177–1190.

Wu Y, Liu J, Yang L, Chen H, Zhang S, Zhao H & Zhang N (2010) Allelopathic control of cyanobacterial blooms by periphyton biofilms. *Environ Microbiol* **13**: 604–615.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. 16S rRNA primers used in this study.
Data S2. Analysis of variance (ANOVA).

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