NITZSCHIA BIZERTENSIS SP. NOV.: A NEW TOXIC DIATOM ISOLATED FROM A SOUTHWESTERN MEDITERRANEAN LAGOON OF BIZERTE

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Abstract

The abundance of toxic diatoms was monthly (November 2007 - February 2009) investigated in the lagoon of Bizerte. These microalgae were present over almost all the sampling period and exhibited a pronounced bloom in August 2008 (3 10 ⁶ cells L⁻¹). The characterization of diatom strains, isolated from seawater, by light/electronic microscopy and molecular analyses allowed to identify a new species *Nitzschia bizertensis*. This specie was characterized by (1) linear-lanceolate valves with 1.38 - 2.91 μm width and 32.52 - 81.73 μm length (2) numerous fibulae in the eccentric raphe and (3) high density of interstriae. The domoic acid (DA) was detected in batch culture of *N. bizertensis* (4500 – 9500 pg DA mL⁻¹). *Nitzschia bizertensis* represents a new domoic acid-producing diatom and is the second toxin producing *Nitzschia* species

Keywords: Diatoms, Lagoons, Toxins, South-Western Mediterranean

Introduction

The occurrence of potentially toxic (PT) diatom blooms has increased in the Bizerte Lagoon [1], which is an important shellfish culture area. Some species of these algae were reported as toxic by of Amnesic Shellfish Poisoning (ASP) and thus may harm human health and represents a serious threat for both aquacultured and wild shellfish. Therefore, we investigated the temporal and spatial dynamics of harmful diatoms in the Bizerte Lagoon. We described species isolated from this area and examinated their toxicity in batch culture.

Materials and Methods

Diatom samples were collected monthly, from November 2007 to February 2009, at several stations in the Bizerte Lagoon, using a plankton net (20 μm diameter). Cell abundance was determined under inverted light microscope [2]. Several strains of PT diatoms were isolated, from seawater, and cultured in f/2 medium [3]. The batch cultures were maintained at a temperature of 20 $^{\circ}$ C, an irradiance of 100 μmol photons m-2 s-1 and a light:dark cycle of 12h:12h. The ultrastructure of the strains was examined using electron microscopy (scanning and transmission) and their genomes were sequenced at LSU and SSU units using the molecular biology techniques. DA in the whole culture (cells plus medium) was analyzed using a the ELISA test.

Results and discussion

During the study, the PT diatoms were abundant in warm, turbid and ammonium-rich waters. These algae exhibited several peaks of density, but the most pronounced bloom was in August 2008 (3 106 cells L-1). Previous studies, in Bizerte Lagoon, showed that PT diatoms were exclusively represented by species of *Pseudo-nitzschia* [1]. In contrast, PT diatoms, isolated during our study, belong to the genus Nitzschia. Cells were described by light and electron (transmission/scanning) microscopy as well as genetically, using the nuclear rDNA regions: SSU, ITS1, 5.8S, ITS2 and D1-D3 of the LSU. The morphological and phylogenetic studies and comparisons with previously described Nitzschia species revealed the presence of a new species: Nitzschia bizertensis sp. nov. The linearlanceolate valve (1.38 - 2.91 μm width and 32.52 - 81.73 μm lengt) was characterized by high density of interstriae (35.1 - 53.2 per 10 µm). The canal raphe was extremely eccentric with a central nodule and contains numerous fibulae (7.6 - 18.6 per 10 μ m). In batch culture, N. bizertensis was able to produce the DA and cellular content varied from 2.3 to 3.6 10⁻² pg DA cell-1. The total DA level varied over time. It was high in exponential growth phase, and thereafter continued to increase during the stationary phase, when it reached 4500 - 9500 pg DA mL⁻¹.

References

1 - Sahraoui I., Bates S.S., Bouchouicha D., Hadj Mabrouk H. and Sakka Hlaili A., 2011. Toxic and potentially toxic *Pseudo-nitzschia* populations in Bizerte Lagoon and first report of domoic acid production by *P. brasiliana* L. *Diat. Res.*, 26: 293-303

- 2 Lund J.W.G., Kipling C. and LeCren E.D., 1958 The inverted microscope method of estimating algal numbers and statistical basis of estimations by counting. *Hydrobiol.*, 11: 143-170
- 3 Guillard R.R.L., 1975. Culture of phytoplankton for feeding marine invertebrates. *In*: Smith, W.L., Chanley, M.H. (eds.), Culture of marine invertebrate animals. Plenum Publishing Corp., New York, pp. 29–60