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Microcystin production in benthic mats of cyanobacteria in the Nile River and irrigation canals, Egypt

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Abstract

The present study describes for the first time the species composition and toxicity of benthic cyanobacteria forming mats on the Nile River and irrigation canal sediments in Egypt. A total of 19 species of cyanobacteria were isolated from these mats during this study. The toxicity of the extracts of these species was investigated using *Artemia salina* assay, mouse bioassay and enzyme linked immunosorbent assay (ELISA). The results showed that all the 19 benthic species isolated from cyanobacterial mats, were toxic to *A. salina*. Two of these species, namely *Calothrix parietina* and *Phormidium tenue*, caused toxicity to mice with neurotoxic signs appeared within 12 h after injection. Whereas, five species showed hepatotoxic effects to mice within 6 h after injection. The results of ELISA showed that all the extracts which had hepatotoxic effects to mice, contained high levels of microcystins with concentrations ranging from 1.6 to 4.1 mg g^{-1} dry weight. HPLC analysis for heptotoxic extracts revealed that these extracts contained two peaks corresponding to microcystin-YR and -LR with different proportions. This study suggests that benthic species should be considered along with planktonic species during monitoring of toxic cyanobacteria in water sources, particularly the Nile river which is the main source of drinking water in Egypt.

Keywords: Benthic cyanobacteria; Mats; Microcystins; The Nile River; Irrigation canals

1. Introduction

Cyanobacteria are known worldwide to produce toxins implicated in animal and human poisoning incidents (Carmichael, 1997; Sivonen and Jones, 1999). Most toxic cyanobacterial species include species of the genera, Anabaena, Aphanizomenon, Cylindrospermopsis, Microcystis, Nodularia, Nostoc and Oscillatoria (Planktothrix) (Skulberg et al., 1993; Lakshmana Rao et al., 2002; Haider et al., 2003). Cyanotoxins cause direct intoxication of animals and humans through contact with water bloom or indirect poisoning due to consumption of contaminated food (Carmichael and Falconer, 1993; Carmichael, 1994, 1997; Jochimsen et al., 1998; Falconer, 1999; Ito et al., 2000; Mohamed, 2001; Mohamed et al., 2003). Cyanotoxins are divided into hepatotoxins (e.g. microcystins, nodularins, cylindrospermopsins), neurotoxins (e.g. anatoxin-a, homoanatoxin-a, saxitoxins) and dermatotoxins (e.g. lyngbyatoxins, aplysiatoxins). Hepatotoxins are inhibitors of serine/threonine specific protein phosphatases (PP1 and PP2A), neurotoxins block neurotransmission and dermatotoxins are the cause of skin irritations, allergic reactions and gastroenteritis (Sivonen, 1996; Carmichael, 2001).

These toxins are found mainly in planktonic cyanobacteria, but a little information is known about the toxicity of benthic species. However, some studies reported that benthic cyanobacteria could produce anatoxins,

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microcystins or saxitoxins (Edwards et al., 1992; Mez et al., 1997; Baker et al., 2001; Hamill, 2001; Verschuren et al., 2002;Gugger et al., 2005).

Most studies on toxic cyanobacteria in Egyptian freshwaters were restricted to planktonic species in the Nile river (Brittain et al., 2000; Mohamed and Carmichael, 2000), but there is no information about the toxicity of benthic cyanobacteria in this country yet. Therefore, the present study aims to predict toxin production in benthic mats of cyanobacteria formed on the Nile river and irrigation canal sediments for the first time in Egypt.

2. Materials and methods

2.1. Sampling

Benthic cyanobacterial mats were collected monthly from sediments at seven sites in irrigation canals (1–7) and seven sites in the Nile river (8–14) at Sohag province (Fig. 1) from January to December 2001.

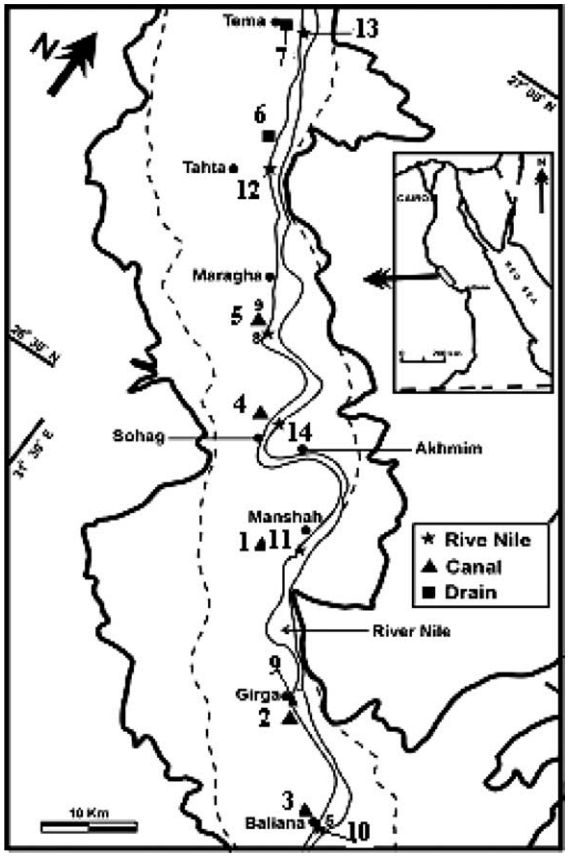

Fig. 1. A map of Egypt showing locations of cyanobacterial mats in irrigation canals and Nile river.

2.2. Identification, isolation and culturing of benthic cyanobacteria

Cyanobacterial species constituting benthic mats on the irrigation canals and Nile river, were identified according to Prescott (1978); John et al. (2002). The single species were isolated and purified by streaking on agar plates containing BG-11 medium (Stanier et al., 1971). The plates were placed at 25 ± 2 °C and irradiance of 24 μmole m^{-2} s^{-1}. After 1 week, the cyanobacterial filaments of the single species were cut at their ends with sterilized forceps, streaked again on the agar plates containing BG-11 medium and grown under the above conditions. To obtain a large quantity of cyanobacetrial species, the single species was transferred from agar plates to a 250 ml-flask containing 100 ml of BG-11 medium and incubated for 1 week. The 100 ml-culture was scaled up by transferring this culture to a flask containing 1 l BG-11 medium. The cultures were then aerated with sterilized air at a rate of 72 l h^{-1}, and the cultures were grown under the same conditions of isolation. The cells were harvested after 3 weeks, and lyophilized.

Table 1
Species composition of cyanobacterial mats collected from the irrigation canals and Nile river during the period of January–December 2001

Species	Irrigation canals	Nile river
Anabaena subcylindrica Borge	++++	++++
A. variablis (Kutz.) Born. Et Flah.	++	+
Calothrix fusca (Kutz.) Born. Et Flah.	+	++
C. parietina (Thur.) Born. Et Flah.	++	+++
Lyngbya epiphytica Heiron.	++++	++++
Nostoc carelum C. Agardh	+	−
Nostoc muscorum C. Agardh	−	++
N. spongioformae (C. Agard.) Born et Flah	−	++++
Oscillatoria angustissima West	++++	++++
O. formosa Gomont	+	++
O. granulate Gardner	+	−
O. limnetica Lemm.	+	++++
Phormidium corium (C. Agard.) Gomont	−	+++
Phormidium tenue (Menegh.) Gomont	+	−
Plectonema boryanum Gomont	+	−
Pseudoanabaena catenata Lauterborn	+++	+++
Rivularia bullata (Poir) Berkeley	+++	−
Scytonema mirabile (Dillwyn) Bornet	+	+
S. myochrous (Dillwyn) C. Agardh	+	−

(++++) Present in all sites; (+++) present in 75% of sites; (++) present in 50% of sites; (+) present in less than of 25% of sites; (−) not present.