Biodiversity of cyanobacteria in Tunisian freshwater reservoirs: occurrence and potent toxicity – a review

Afef Fathalli, Mohamed S. Romdhane, Vitor Vasconcelos and Amel Ben Rejeb Jenhani

ABSTRACT

In spite of the great environmental and sanitary importance of cyanobacteria, their biodiversity is little known in Tunisia. In this work, a review was carried out, based on literature data, of potentially toxic cyanobacteria occurrence in Tunisia. *Microcystis,* one of the most widely distributed toxic bloom-forming cyanobacteria genera, was represented by *Microcytis wesenbergii,* found only in Lebna water reservoir, and *Microcytis aeruginosa* recorded in different water bodies. The invasive potentially toxic cyanobacterium *Cylindrospermopsis raciborskii*, reported for the first time in Tunisia in October 2004, was observed in the semi-arid to arid areas. The harmful genus *Planktothrix* was represented in Tunisian freshwater by the green-pigmented species *P. agardhii*. The filamentous cyanobacteria dominance is increasingly reported in Tunisia in eutrophic water bodies. This dominance increases especially during the summer–autumn period. Recently, potentially toxic cyanobacteria blooms have been reported in some reservoirs in the north east of the country. These blooms were generated by the potentially toxic Chroococcale *Microcystis aeruginosa.* Harmful cyanobacteria tend to spatio-temporal expansion in the Tunisian inland waters. The toxicological potential evaluated by several methods showed that none of the Tunisian strains were proved to be cylindrospermopsin nor saxitoxin producers. However, the majority of *Microcystis* were able to synthesize microcystin.

Key words | cyanobacteria, cyanotoxins, genetic variability, Tunisian freshwaters

Afef Fathalli (correspoding author)
Mohamed S. Romdhane
Amel Ben Rejeb Jenhani
Unité de Recherche Ecosystèmes et Ressources Aquatiques,
Institut National Agronomique de Tunisie,
43, Avenue Charles-Nicolle,
1082 Tunis Mahrajène,
Tunisia
E-mail: *fathalli_afef@yahoo.fr*

Afef Fathalli
Institut National des Sciences et Technologie de la Mer, Port de pèche-2060 La Goulette, Tunis,
Tunisia

Vitor Vasconcelos
Centro de Investigação Marinha e Ambiental-CIIMAR/CIMAR, Universidade do Porto,
Rua dos Bragas, 289,
4050–123 Porto, Portugal
and
Departamento de Biologia, Faculdade de Ciências,
Universidade do Porto,
Rua do Campo Alegre,
4169–007 Porto,
Portugal

INTRODUCTION

Cyanobacteria are a major group of prokaryotes that occur throughout the world (WHO 1998). They are the Earth's oldest known oxygen-producing organisms, with fossil remains dating back 3500 million years (Schopf 2000). Through their photosynthetic activity, they were largely responsible for the modern-day oxygen-enriched atmosphere, and subsequent evolution of our planet's higher plant and animal life (Schopf 2000; Whitton & Potts 2000). Cyanobacteria have many unique features among phytoplankton, such as buoyancy and nitrogen fixation, and the production of a wide variety of bioactive compounds. Several species of cyanobacteria form blooms that are frequently toxic, and thus pose a health risk for humans and animals (Sivonen & Jones 1999). They can produce toxic secondary metabolites including hepatotoxins that have carcinogenic potential, neurotoxins and lipopolysaccaride endotoxins (Carmichael & Falconer 1993; Codd 2000; Carmichael 2001). The tragic deaths of 70 of 131 patients exposed to the hepatotoxins microcystins (MC) through renal dialysis in Brazil are the only well known substantiated human fatalities due to cyanotoxins (Jochimsen *et al.* 1998). Nevertheless, some illnesses reported previously were life-threatening (Ressom *et al.* 1994), such as the poisoning of 138 children and 10 adults due to hepatotoxin cylindrospermopsin (CYN) in Palm Island (Australia) (Hawkins *et al.* 1985).

While cyanobacterial harmful algal blooms have been reported in the scientific literature for more than 130 years (Francis 1878), in recent decades, the incidence and intensity

doi: 10.2166/aqua.2015.119

of these blooms, as well as economic loss associated with these events, has increased in both fresh and marine waters (Chorus & Bartram 1999; Carmichael 2001; Hoagland *et al.* 2002; Teneva *et al.* 2005; Hudnell 2008; Heisler *et al.* 2008; Paerl 2008; Paul 2008; Paerl & Huisman 2008). The most common cyanobacterial genera known for their potential ability to produce toxins include *Anabaena, Aphanizomenon, Cylindrospermopsis, Lyngbya, Microcystis, Nodularia, Nostoc* and *Planktothrix*. However, the number of identified toxic cyanobacteria is still increasing as a result of new detections (Sivonen 1998; Aboal & Puig 2005; Humpage *et al.* 2012). In fact, Ballot *et al.* (2005) reported that the hepatotoxin MC-YR and the neurotoxin anatoxin-a were produced, in a monocyanobacterial strain of *Arthrospira fusiformis*, isolated from Lake Sonachi (Kenya). It is important to note that the cyanobacterium *Arthrospira (Spirulina)*, having a long history of use as food for humans (Vonshak 1997), is used as a nutritive supplement for its beneficial effects, including detoxication, increased energy, weight loss and therapeutic applications (Ciferri 1983; Belay 1997; Mei Li & Zao Qi 1997). *Limnothrix*, a commonly occurring cyanobacterial genus, was recently shown to produce a novel toxin '*Limnothrix*i' (Torres-Ariño & Mora-Heredia 2010; Bernard *et al.* 2011).

The dominance of cyanobacteria in aquatic ecosystems, in different geographical locations, with considerable variability in terms of size, morphology, salinity and hydrologic conditions, indicates that climate change can help synergistically with anthropogenic nutrient enrichment on the growth of these micro-organisms across the globe (Paerl & Paul 2012). Several studies reported that freshwater cyanobacteria blooms are typically associated with eutrophic and poorly flushed waters (Paerl 1988; Carmichael 1995; Lee *et al.* 2000; Albay *et al.* 2003). Kosten *et al.* (2012) conducted monitoring in 143 lakes of different trophic situations, localized according to the latitudinal gradient from north of Europe to South America. They found that the percentage contribution of cyanobacteria in total phytoplankton biomass increases significantly with temperature. These authors noted also that the rise in temperature can reduce the levels of nutrient uptake that cause the initiation and maintenance of blooms. Recent studies in laboratory scale as well as field observations have clearly demonstrated that the combination of anthropogenic inputs of nutrients, rising temperatures, increased stratification and elevated atmospheric CO_2 concentrations, favored the dominance of cyanobacteria in a wide range of aquatic ecosystems (Paerl & Paul 2012). The warm Mediterranean climate favors the occurrence and extended duration of the cyanobacterial blooms in eutrophic freshwaters, which may start in spring and persist until December, or in hypertrophic lakes, may even be continuous throughout the year (Moustaka-Gouni *et al.* 2007). Although toxic and non-toxic strains usually coexist in a water body, distinguishing toxic strains from non-toxic strains is impossible under a microscope. Therefore, many studies have focused on the discriminative detection of toxin-producing strains (Baker *et al.* 2001; Via-Ordorika *et al.* 2004; Ouahid *et al.* 2005). Researchers have already described the MC-producing mechanism on the genetic level (Dittmann *et al.* 1997; Tillett *et al.* 2000). MC are produced by the enzyme complex MC synthetase encoded in the *mcy* gene cluster, which code for both non-ribosomal peptide synthetases (PSs) and polyketide synthases (PKSs) (Dittmann *et al.* 2001). *Microcystis mcy* genes are organized in a cluster of two operons. One operon contains PS genes (*mcyA*, *mcyB*, *mcyC*), responsible for the incorporation and activation of the amino acid [MDha-Ala-X-MAsp-Z] constituents of MC. The second operon includes one PKS gene (*mcyD*), two hybrid genes corresponding to PS and PKS (*mcyE* and *mcyG*), and additional genes (Nishizawa *et al.* 2000). *McyD*, *-G* and *-E* genes code for the Adda group synthesis. The association of glutamic acid with the Adda group and its activation are encoded by the *mcyE* gene (Sivonen & Börner 2008). Kellmann & Neilan (2007) have proposed the saxitoxin (STX) biosynthesis pathway. The *sxt* gene clusters within each organism all contain a core set of genes putatively responsible for the biosynthesis of STX. The production cylindrospermopsin (CYN) implicates enzymes coded by the PS and the PKS genes. Schembri *et al.* (2001) described a novel amidinotransferase (AMT) gene within the genomic region encoding PS and PKS. Fergusson & Saint (2003) developed a multiplex-polymerase chain reaction (PCR) test to amplify PS and PKS determinants associated with CYN production and to distinguish *Cylindrospermopsis raciborskii* strains from other CYN-producing cyanobacteria. The discovery of these genes will allow the rapid and accurate detection of harmful paralytic shellfish poisoning (PSP)-producing species in water (Kellmann *et al.* 2008).

TUNISIAN CONTEXT

Tunisia is a Mediterranean country located in the northeastern extremity of Africa. With Sicily, it defines both eastern and western basins of the Mediterranean. It covers an area of 164,150 km². Being under the disruptive climatic influence of the temperate region in the north and the Saharan region in the south, Tunisia is characterized by relative aridity on the major part of its territory. Indeed, two-thirds of the country receives rainfall amounts between 50 and 350 mm/year (Ben Boubaker *et al.* 2003). To this aridity is added the variability of the Mediterranean climate, with erratic and unpredictable periods of drought and violent floods, to make water an often limited resource and distributed unequally in time and space (Benzarti 2003). Bergaoui & Louati (2010) showed the temperature increase with an inflow reduction even for relatively wet hydrological years. Tunisia's hydric potential, mainly formed by surface water, is very modest. It was estimated at 4,630 thousand million m^3. It is the lowest value of the Maghreb countries (Ben Boubaker *et al.* 2003). In fact, the important evaporation which is the cause of the climatic water balance to be in deficit, and the reduced size of the catchment areas make Tunisia one of the Mediterranean countries less endowed with hydraulic resources. The water resource volume available per capita is 450 m^3/year against 556 m^3/year in Morocco, 776 m^3/year in Syria and 2,200 m^3/year in Turkey (Cherif 2003).

Tunisia's freshwater resources are under increasing stress from a growing population and an expanding economy. In addition, almost all of the country's freshwater resources have been fully allocated, while the water quality of these resources has declined due to increased pollution caused by industry, urbanization and agriculture. The reservoirs have become progressively more enriched during recent decades. The importance and current extent of eutrophication in Tunisian water bodies has been highlighted in previous studies, showing that these ecosystems present an increasing productivity stimulated continually by fertilizing contributions owing to the important anthropisation and the drier climate (Mouelhi 2000; Turki 2002; Fathalli *et al.* 2006; El Herry *et al.* 2008a). Eutrophication is generally indicated by high values of nutrients, accumulation of metabolic products (e.g., hydrogen sulfide in deep waters), discoloration or turbidity of water (resulting in low or poor light penetration), deterioration in the taste of water, depletion of dissolved oxygen and an enhanced occurrence of toxic cyanobacterial bloom-forming species (Dauta & Feuillade 1995). However, in spite of its great environmental and sanitary importance, the biodiversity of cyanobacteria is little known in Tunisia as a consequence of a small number of publications. In fact, the first reference to Tunisian freshwater toxic cyanobacreria was published only in 2006 (Ben Rejeb Jenhani *et al.* 2006).

In this review, we report the available information on the presence of potentially toxic cyanobacteria in Tunisian water storage reservoirs that have already been subject of limnological studies.

OCCURRENCE OF POTENTIALLY TOXIC CYANOBACTERIA IN TUNISIAN FRESHWATER

Data were obtained from scientific articles, reports, theses and communications at congresses. The water bodies are located in the four most important Tunisian hydrological basins (Figure 1, Table 1).

Investigations conducted in Tunisian reservoirs revealed the presence of 55 species of cyanobacteria belonging mainly to filamentous strains. In fact, these species are represented by 34 Oscillatoriales, 16 Chroococcales, three Nostocales and two Pleurocapsales. Some of them have been described in the literature as potentially toxic (Table 2). The identification of the majority raw cyanobacterial samples was performed using UTERMÖHL technique based on classic inverted microscopy. However, some of them are confirmed by molecular biology tools using isolated and cultured strains (El Herry *et al.* 2008a; 2008b; Fathalli *et al.* 2010, 2011a, 2011b). In fact, molecular taxonomy proved necessary in order to avoid confusion between morphologically similar species.

Microcystis, one of the most widely distributed toxic bloom-forming cyanobacteria genera, was observed in 58% of studied Tunisian reservoirs. It was represented by *Microcystis wesenbergii,* found only in the Lebna dam, and *Microcytis aeruginosa* recorded in different water bodies from different geographic situations across the country (Figure 2) (El

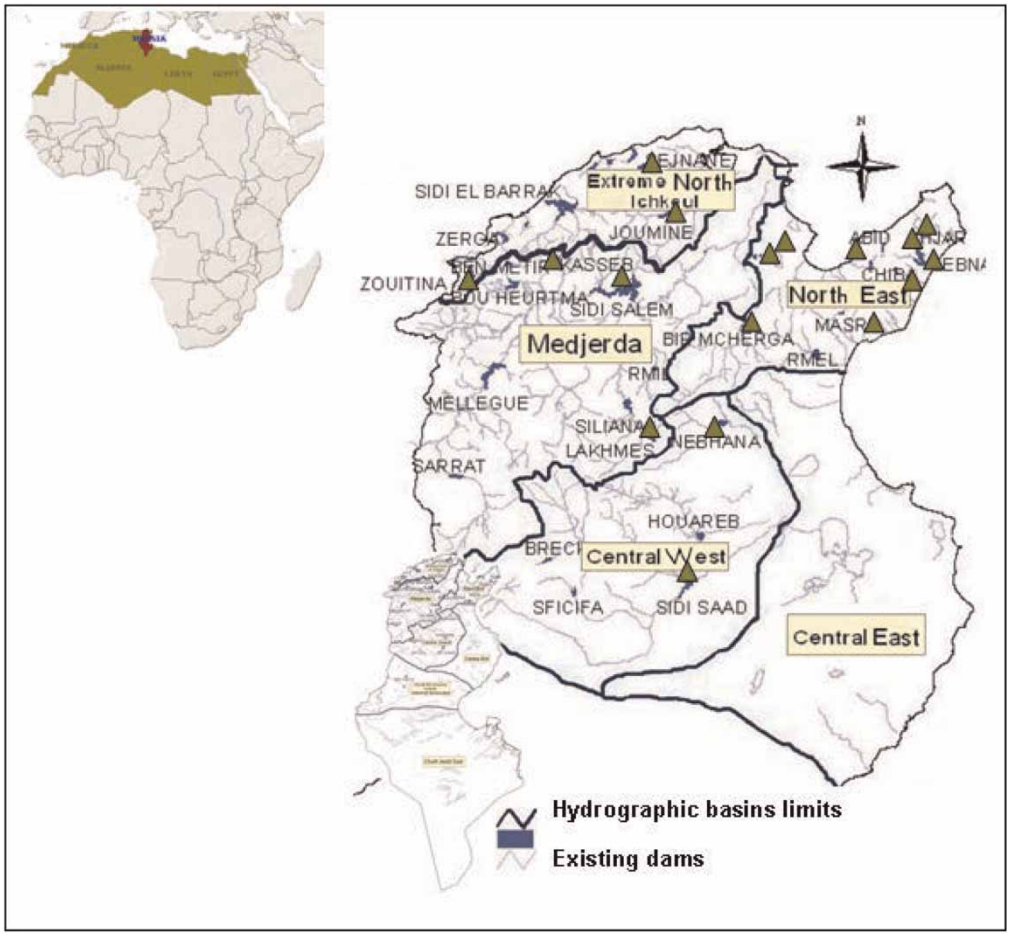

Figure 1 | Tunisian hydrological basins and the location of major dams (Ben Mammou and Louati 2007).

Herry *et al.* 2008a; Fathalli *et al.*, 2011a; Sellami *et al.* 2012). This latter species was the most incriminated cyanobacterium in incidents of human and animal poisoning over the world by production of the hepatotoxins MC that present over 70 natural structural variants, and are potent and specific inhibitors of protein phosphatases (Chorus & Bartram 1999; Codd *et al.* 2005). Based on morphological criteria, 10 species have been distinguished in Europe, the other side of the Mediterranean: *Microcystis aeruginosa, M. viridis, M. wesenbergii, M. novacekii, M. ichthyoblabe, M. flos-aquae, M. natans, M. firma, M. smithii* and *M. botrys* (Komárek & Anagnostidis 1999). In North African freshwater bodies, four species are found: *Microcystis aeruginosa, M. wesenbergii, M. ichthyoblabe* and *M. novacekii* (Abdel-Rahman *et al.* 1993; Oudra *et al.* 2001; Oudra *et al.* 2002; Nasri *et al.* 2004; Ben Rejeb Jenhani *et al.* 2006; El Herry *et al.* 2009).

The invasive potentially toxic freshwater cyanobacterium *Cylindrospermopsis raciborskii*, which was firstly recorded in tropical to subtropical climate regions, was reported for the first time in Tunisia in October 2004 (Fathalli *et al.* 2010). It was observed in freshwater Bir M'cherga reservoir as pale blue-green, straight trichomes without mucilaginous sheaths, bearing terminal drop-shaped heterocysts with pointed ends. Coiled trichomes were never observed in Tunisian freshwater. This species was observed, later, in Nebhana and Sidi Saâd reservoirs, located in the center of the country (Fathalli *et al.*, 2011a). These reservoirs are characterized by relatively high trophic levels (Fathalli *et al.* 2010; Sellami *et al.* 2010, 2012) and their environmental conditions seemed to be favorable for the species appearance. Fathalli *et al.* (2010) showed that the *Cylindrospermopsis* density was correlated positively with

Table 1 | Characteristics and uses of some water bodies in Tunisia

Dams	Geographic coordinates	Impound-ment	Main supply	catchment area (Km²)	Volume (10^6 m³)	Area (Ha)	Min-max or means T (°C)	NO_3^- (mg/l)	PO_4^{3-} (mg/l)	Uses
S	N37°10′42″ E09°28′25″	1994	Séjnène river	376	138	790	18.56	2.25 – 5.,88	-	Drinking water; irrigation; fish farming
J	N36°59′23″ E09°36′59″	1983	Joumine river	418	130	660	19.3	2.52 – 5.34	0.01 – 0.29	
H	N36°52′ E11°02′	1996	El Hjar river	61	5	254	12.3–28.8	7.05	0.031	Irrigation
B	N 36°43′16″ E 10°37′57″	1959	Bezirk river	84	6.5	102	13.2–31.2	3.02–8.24	0.00–0.08	
Ml	N 36° 49′ 27″ N10° 59′ 26″	1965	Mlaabi river	35	3.7		13.2–29.4	0.42–4.23	0.00-0.09	
Ch	N 36°41′52″ E 10°46′17″	1963	Chiba river	63	6.9	66	12.3–28.3	1.35–6.86	0.00–0.08	
M	N 36°31′50″ E 10°29′08″	1968	Masri river	53	6.9	66	12.1–30	2.35–9.60	0.00–0.07	Drinking water; irrigation
BMt	N 36°43′10″ E 08°44′10″	1953	El lil river	103	57.6	310.6	-	-	-	Drinking water irrigation; fish farming electric energy
K	N 36°45′30″ E 09°00′50″	1968	Kasseb river	101	81	437	21,4	2,22	0,02	
SSM	N36°35′27″ E 09°23′51″	1981	Medjerda river	18,000	555	4,300	10.2 – 27.4	0.12 – 2.88	0.001 – 0.056	
GG	N 36° 44′ E 09° 58′ 50″	1968	Kasseb reservoir Medjerda/Cap Bon channel	-	3	40	12.5–29.5	0.35–5.96	0.005–0.042	Drinking water
Mo	N 36° 44′ E 09° 58′ 50″	1982	Kasseb reservoir Medjerda/Cap Bon channel	-	15	150	12.5–29.5	0.22–6.35	0.00–0.035	
L	N36°44′01″ E10°55′08″	1986	Lebna river	189	30.2	650	19,7	0.72	0.09	Irrigation; fish farming
LK	N 35°59′58″ E 09°28′31″	1966	Lakhmess river	127	8	102	-	-	-	
BM	N36°30′36″ E10°00′38″	1971	Méliane river	1,260	209	2,000	20.3	1.69	0.16	
N	N36°03′34″ E09°52′34″	1965	Nebhana river	855	87.2	532	22.04	2.34	0.09	
SS	N35°25′31″ E09°41′50″	1981	Zeroud river	8,950	53	110	8.7 – 29	0.04 – 0.43	0.03	

S: Séjnène; J: Joumine ; H: Hjar; L: Lebna; B: Bezirk ; M: Masri; Ml: Mlaabi; Ch: Chiba; BMt: Beni Mtir; K: Kasseb; SSM: Sidi Salem; GG: Gdir el golla; Mo: Mornaguia; LK: Lakhmess; BM: Bir M'cherga; NB: Nebhana; SS: Sidi Saâ.

temperature, transparency and salinity. In fact, the occurrence of *C. raciborskii* was recorded in summer when the temperature values were higher than 20 °C. This confirms previous observations (Padisák 1997; Briand *et al.* 2002) showing that a water temperature ranging between 22 and 23.5 °C is a key factor for the germination of akinetes. The presence of this species during this period was also supported by the decrease of nitrate values (Fathalli *et al.* 2010). The same result was found by Briand *et al.* (2002), who observed a low nitrate concentration during the

Table 2 | Inventory cyanobacteria species in Tunisian reservoirs

Order	Species	S	J	H	L	B	M	Ml	Ch	BMt	K	SSM	GG	Mo	LK	BM	NB	SS
Chroococcales	*Aphanothece c.v. brevis*											x						
	Merismopedia glauca	x	x	x	x	x	x	x	x					x				
	Merismopedia elegans										x	x		x				
	Merismopedia miniata											x						
	Merismopedia sp.															x		
	Microcytis aeruginasa	x●	x●	x●	x●					x		x			x	x●	x●	x
	Microcytis wesenbergii				x●													
	Chroococcus sp.			x	x	x	x	x	x							x	x	x
	Chroococcus minitus												x					
	Chrococcus turgidus				x													
	Aphanothece sp.												x					
	Aphanocapsa muscicola												x					
	Snowella sp.				x													
	Snowella atomus				x													
	Synechococcus elongatus										x					x		
	Synechocystis aqualis				x													
Nostocales	*Anabaena sp.*			x	x			x				x						x
	Aphanizomenon sp.															x		
	Cylindrospermopsis raciborskii															x●	x●	x●
Oscillatoriales	*Borzia trilocularis*				x			x			x							
	Limnothrix sp.															x●		
	Limnothrix redekei																	x●
	Leptolyngbya sp.																x●	
	Lyngbya sp.										x	x				x		
	Lyngbya limnetica												x	x				
	Lyngbya rubida										x							
	Oscillatoria acutissima													x				
	Oscillatoria amphibia													x				
	Oscillatoria articulata	x	x															
	Oscillatoria chlorina	x			x			x				x	x	x		x		
	Oscillatoria geminata											x						
	Oscillatoria homogenea	x	x								x					x	x	x
	Oscillatoria lacustris										x	x				x		x
	Oscillatoria limnitica	x	x		x													
	Oscillatoria planctonica	x		x	x		x	x				x				x		x
	Oscillatoria pseudogeminata										x					x		
	Oscillatori simplissima				x													
	Oscillatoria splendida												x	x				
	Oscillatoria spp.	x	x	x	x	x	x	x	x		x	x		x		x		x
	Oscillatoria tenuis				x●						x	x				x		
	Phormidium cf incrustatum										x			x				

(*continued*)

Table 2 | continued

Order	Species	S	J	H	L	B	M	Ml	Ch	BMt	K	SSM	GG	Mo	LK	BM	NB	SS
	Phormidium frigidum													X				
	Phormidium luridum												X	X				
	Phormidium olivascens												X	X				
	Phormidium vezii													X				
	Phormidium sp.	X			X						X		X					
	Phormidium tenue												X	X				
	Planktolyngbya subtilis																	X
	Planktothrix agardhii		X●													X●	X●	X●
	Planktothrix mougeotii													X				
	Pseudanabaena catenata	X		X	X	X		X			X	X	X	X		X		X
	Pseudanabaena constricta				X						X	X				X	X	
	Romaria sp.																X	
Pleurocapsales	*Hydrococcus sp.*					X												
	Hydrococcus rivularis				X						X							

x: species identified by the classical method of inverted microscopy; ●: species confirmed by the molecular biology tools.
S: Séjnène; J: Joumine ; H: Hjar; L: Lebna; B: Bezirk ; M: Masri; Ml: Mlaabi; Ch: Chiba; BMt: Beni Mtir; K: Kasseb; SSM: Sidi Salem; GG: Gdir el golla; Mo: Mornaguia; LK: Lakhmess; BM: Bir M'cherga; NB: Nebhana; SS: Sidi Saâd.
List established from following references (Ben Rejeb Jenhani 1990; Kümmerlin 1991; Ben Rejeb Jenhani *et al.* 2006; Fathalli *et al.* 2006; INSTM 2006; Romdhane *et al.* 2006; El Herry *et al.* 2008a; Fathalli *et al.* 2011a; Fathalli 2012; Sellami *et al.* 2012).

summer proliferation. Chorus & Bartram (1999) reported that the lack of nitrate was considered to be the main reason for the proliferation of heterocystic species, such as *C. raciborskii*.

Hepatotoxin-producing strains of *C. raciborskii* have been found in Europe, Australia, Asia and Africa (Hawkins *et al.* 1997; Saker *et al.* 1999, 2003; Li *et al.* 2001; Fastner *et al.* 2003; Bernard *et al.* 2003; Mohamed 2007), while neurotoxin-producing strains have been reported in Brazil, where it produced PSP toxin (Lagos *et al.* 1999). Incidents where *C. raciborskii* has been suspected to cause human sickness (Bourke *et al.* 1983; Hawkins *et al.* 1985) and cattle mortality (Saker *et al.* 1999) have been restricted to Australia. This toxic effect is now known to be due to the alkaloid CYN (Terao *et al.* 1994).

In Africa, the occurrence of *C. raciborskii* has also been recorded in Algeria, Egypt, Uganda, Senegal and South Africa (Bouaïcha & Nasri 2004; Mohamed 2007; Haande *et al.* 2008; Janse van Vuuren & Kriel 2008). From all these strains, only the Egyptian one was reported as hepatotoxic by the mouse bioassay (Mohamed 2007). *C. raciborskii* has been observed in other Mediterranean countries, including Spain, France, Italy, Greece and Israel (Romo & Miracle 1994; Bernard *et al.* 2003; Vardaka *et al.* 2005; Moustaka-Gouni *et al.* 2007; Messineo *et al.* 2010; Alster *et al.* 2010).

Planktothrix, considered to be an important genus of harmful cyanobacteria since it is one of the most frequent MC producers (Kurmayer *et al.* 2005), was represented in Tunisian freshwater by the green-pigmented species *P. agardhii*. This genus may contain a higher concentration of MC per cell than *Microcystis*. So, the risk level is uppermost when *Planktothrix* is dominant (Codd *et al.* 2005). The species *P. agardhii* was recorded at four different reservoirs (Joumine, Bir m'cherga, Nebhana and Sidi Saâd). Fathalli (2012) reports that, from 1 year to another, there is a considerable development increase of the species. Indeed the average density, in Bir M'cherga dam, changes from 0.017×10^6 filaments/l in 2005 to 0.5×10^6 filaments/l in 2006. Moreover, the same author notes a very high density of *P. agardhii* at the beginning of 2007 reaching 6.69×10^6 filaments/l. The species *P. agardhii* has generally presented a relatively homogeneous vertical distribution. It is endowed with dispersed development ecostrategy (Chorus & Bartram 1999). Despite its preference for warm waters (Berger &

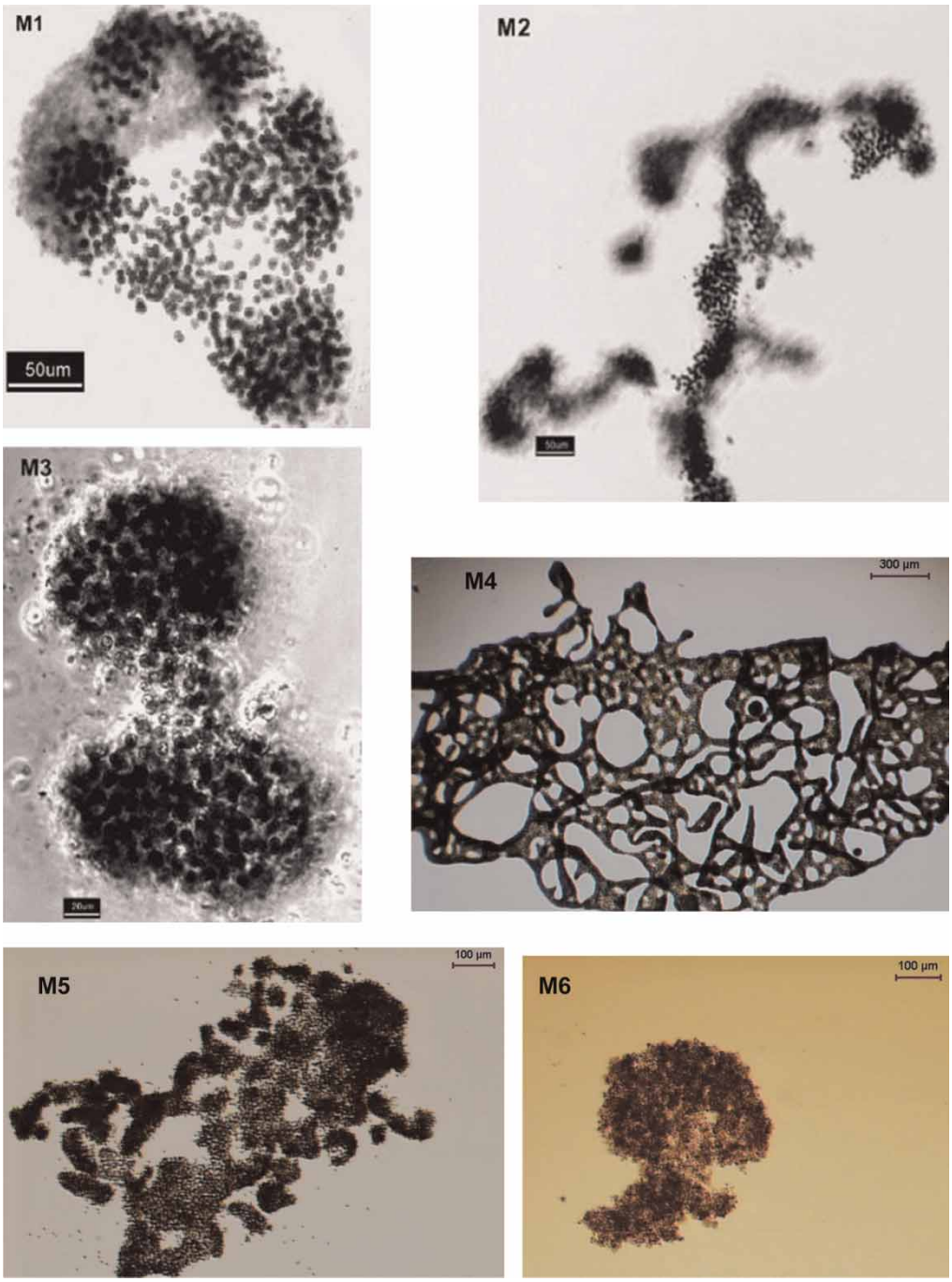

Figure 2 | Toxic strains of *Microcystis* observed in Tunisian freshwater (El Herry *et al*. 2008a; Fathalli 2012). M1, M2: *M. aeruginosa* occurred in Lebna reservoir; M3: *M. wesenbergii* occurred in Lebna reservoir; Micro-NB-01: *M. aeruginosa* isolated from Nabhena reservoir; Micro-JM-02: *M. aeruginosa* isolated from Joumine reservoir; Micro-HJ-02: *M. aeruginosa* isolated from Hjar reservoir.

Sweers 1988), this species can occur in high densities throughout the year thanks to its tolerance for low temperatures and low light intensities (Dokulil & Teubner 2000; Scheffer, 1998). Thus, a competitive advantage is expressed in taxa that tolerate low light intensities, i.e., *Planktothrix*, *Cylindrospermopsis raciborskii* and *Limnothrix*, which

thrive better than the species susceptible to loss of light, i.e., *Anabaena spp.* and *Aphanizomenon spp.* (Reynolds *et al.* 2002). In fact, an assemblage of these three filamentous cyanobacteria (Figure 3) (*Planktothrix agardhii*, *Cylindrospermopsis raciborskii* and *Limnothrix sp.*) was reported in the freshwater Bir M'cherga dam (Fathalli 2012).

The filamentous cyanobacteria dominance is increasingly reported in Tunisia in eutrophic water bodies such as Sidi Saâd (Sellami *et al.* 2012) and Bir M'cherga dams (Fathalli *et al.* 2010). This dominance increases especially during the summer–autumn period. Indeed, Fathalli (2012) showed the persistence of high cyanobacteria density in freshwater Bir M'cherga reservoir throughout the year. Upper densities of 6.14×10^6 and 9.58×10^6 individuals per liter were recorded during July 2005 and 2006, respectively. Recently, potentially toxic cyanobacteria blooms have been reported in some reservoirs in the north (Hjar and Lebna dams). These blooms were generated by the potentially toxic Chroococcale *Microcystis aeruginosa* (Ben Rejeb Jenhani *et al.* 2010). Oudra *et al.* (2001), Sabour *et al.* (2002), Nasri *et al.* (2004, 2008) and Douma *et al.* (2010) showed that in Morocco and in Algeria, a neighboring country with close climatic conditions, natural cyanobacterial blooms containing MC were dominated by the genus *Microcystis*. Thus, harmful cyanobacteria tend to spatio-temporal expansion in the Tunisian inland waters. They are consequently considered as precursors of freshwater-quality degradation.

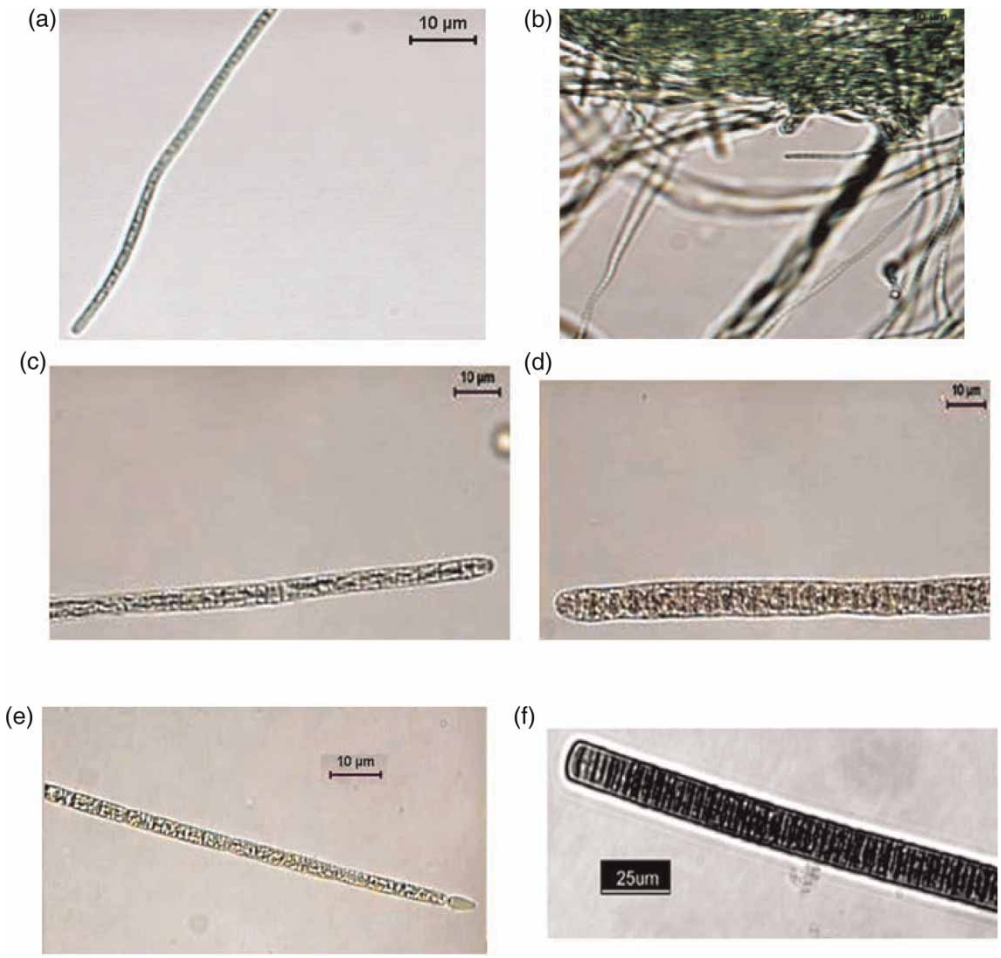

Figure 3 | Some filamentous cyanobacteria strains observed in Tunisian freshwater (El Herry *et al*. 2008a; Fathalli 2012). (a): *Limnothrix sp.* isolated from Bir M'cherga reservoir; (b): *Leptolyngbya sp.* isolated from Nabhena reservoir; (c): *Planktothrix agardhii* isolated from Bir M'cherga reservoir; (d): *P. agardhii* isolated from Joumine reservoir; (e): *Cylindrospermopsis raciborskii* isolated from Bir M'cherga reservoir; (f): *Oscillatoria tenuis* occurred in Lebna reservoir.

OCCURRENCE OF CYANOBACTERIAL TOXINS IN TUNISIAN FRESHWATER

While the occurrence of cyanotoxins was reported worldwide since the beginning of the last century (Sivonen & Jones 1999), in Tunisian freshwater, their presence has been confirmed only in 2003, from Hjar Reservoir in the Cap Bon region, North-East of the country, using high performance liquid chromatography coupled to a diode array detector and tandem mass spectrometry technique that revealed three variants of the hepatotoxins MC: MC-LR, MC-RR, MC-YR (El Herry *et al.* 2007). The cyanotoxicity monitoring of the Tunisian freshwater was performed mainly using the protein phosphatase (PP2A) inhibition assay (Table 3). Ben Rejeb Jenhani *et al.* (2006) demonstrated that, compared to other untreated water, those of Hjar, Mlaabi and Lebna dams are distinguished by their higher levels of toxins, often observed in the particulate fraction. The same authors report that the maxima observed in these water bodies have coincided with a relatively massive development of *Oscillatoria* and *Pseudoanabaena* genera in the Hjar dam and the species *Oscillatoria chlorina* in Mlaabi freshwater. El Herry *et al.* (2008a) noted that toxin concentrations reached a value of 5.57 μg MC-LR equivalent per liter in the Lebna reservoir suggesting that the three morphospecies of *Microcystis* and the filamentous species *O. tenuis* that occurred in this dam are potentially toxigenic. In this water body two variants of MC (MC-LR and MC-YR), were identified. This finding is certainly consistent with prior studies from Mediterranean countries, which have shown that MC-LR is the major toxin in cyanobacterial blooms from France (Vezie *et al.* 1998), and Morocco (Oudra *et al.* 2002) and frequently co-occurs with MC-YR and/or MC-RR in Morocco (Oudra *et al.* 2001), in Algeria (Nasri *et al.* 2004; 2008), and in Turkey (Albay *et al.* 2005). The cyanotoxins concentrations detected in the three above-mentioned dams, used mainly for irrigation, was higher than the guideline value for MC-LR (1 μg L^{-1}) established by the WHO (1998) for drinking water. However, they remain lower than those found in Algeria (Nasri *et al.* 2004) and in Morocco (Oudra *et al.* 2001), where the MC concentrations were estimated to be 29,163 μg MC-LR equivalent L^{-1} and >500 μg MC-LR equivalent L^{-1}. Saqrane & Oudra (2009) reported that cyanotoxicity impact on both aquatic and terrestrial crop plants irrigated by water containing these toxins has become more and more available. This fact is gaining importance since plants could in a direct or indirect manner contribute to cyanotoxin transfer through the trophic network, and thus constitutes a potent health risk source. The use of this contaminated irrigation water can also have an economic impact which appears as a reduction in the germination rate of seeds, and alteration of the quality and the productivity of crop plants (Silva & Vasconcelos 2010).

Table 3 | Extreme values of cynotoxins concentrations in Tunisian freshwaters (using PP2A inhibition assay)

	Total cyanotoxins (μg MC-LR equivalent L^{-1})	References
Ghdir el Golla	0.001–0.028	Ben Rejeb Jenhani *et al.* (2006)
Mornaguia	0–0.024	
Sejnene	0.001–0.837	
Joumine	0.001–0.168	
Hjar	0.023–7.455	
Bezirk	0.011–0.500	
Masri	0.032–0.752	
Mlaabi	0.010–1.040	
Chiba	0.003–0.495	
Kasseb	0–0.034	Fathalli *et al.* (2006)
Lebna	0.021–5.485	El Herry *et al.* (2008a)
Bir M'cherga	0.002–0.931	Fathalli *et al.* (2010)

The Tunisian freshwaters studied were classified according to three levels of toxicity: <0.1 μg MC-LR equivalent L^{-1}, 0.1–1 μg MC-LR equivalent L^{-1} and >1 μg MC-LR equivalent L^{-1}. All Tunisian reservoirs used for drinking water production have been characterized by toxicity values less than 1 μg MC-LR equivalent L^{-1} (Ben Rejeb Jenhani *et al.* 2006). The highest values of toxicity were recorded at dams situated in the north-east hydrologic basin characterized by high anthropogenic activity. In fact, this region which represents 8.5% of the whole area of Tunisia, concentrates paradoxically 45% of urban population, 49% of industrial employment, 36% of tourism capacity and 30% of irrigable surface (Cherif 2003).

GENOMIC POTENTIALITY OF CYANOTOXINS SYNTHESIS

The cyanotoxins have been reported in almost the whole regions where cyanobacteria were studied, thus making the cyanotoxicose a public health problem of global

concern. Cyanobacterial toxins are usually classified according to their mode of action, into hepatotoxins (MC, nodularins and cylindrospermopsins), neurotoxins such as the STXs, and dermatotoxins which is characterized by their lipopolysaccharide nature (Chorus & Bartram 1999). The toxic and non-toxic character of the same species may vary between different strains (Carmichael 1992). Recent development of techniques in biotechnology has enabled the development of new methods of molecular detection of cyanobacteria and their toxins. Detection methods based on PCR amplification of DNA of Cyanobacteria are interesting because of their potential selectivity with respect to genes involved in the biosynthesis of toxins, their sensitivity and timeliness (Ouellette & Wilhelm 2003). In Tunisia, few studies have been conducted on the biodiversity of cyanobacteria and their molecular characterizations (Table 4). El Herry *et al.* (2008a) showed the expression of the NMT domain of the MC synthetase genes *mcyA*, *-B* and *-C* in the genome of three morphospecies of *Microcystis* and the filamentous species *Oscillatoria tenuis* collected from the natural samples of Lebna reservoir. The authors indicated that these cyanobacterial species were responsible for MC production in this dam. They confirmed this result through the detection of MC-LR and MC-YR, in the cyanobacterial sample containing the three morphospecies of *Microcystis* and the filamentous species *O. tenuis,* via LC/MS/MS technique. Fathalli *et al.* (2011a) evaluated, also by molecular biology tools, the toxicological potential of 27 cyanobacterial strains isolated from seven reservoirs located in the north and center of Tunisia and belonged mainly to *Microcystis aeruginosa*, *Cylindrospermopsis raciborskii* and *Planktothrix agardhii* species. This study showed that none of the isolated strains carried segments of the gene cluster responsible for the production of cylindrospermopsin and STX, while the majority of *Microcystis* isolates were able to synthesize MC, since they presented the six characteristic segments of the MC synthetase mcy cluster (*mcyA,-B,-C,-D,-E* and-*G*). This was further confirmed by MALDI-TOF analysis that showed the presence of eight MC variants, including MC-LR. *Microcystis* strains isolated from Lebna (Micro-LB-01) and Bir M'cherga (Micro-BM-02) reservoirs did not produce any kind of MC. In fact, these strains showed the absence of either the gene *mcyA* or *mcyA,-C,-D* and-*E*. In another study, Fathalli *et al.* (2011b) assessed the toxicity of four *C. raciborskii* strains isolated from the Bir M'cherga Tunisian reservoir. They concluded that all the isolated strains were not producing cylindrospermopsin, STX or MC. This result was further confirmed by HPLC and MALDI-TOF analyses. However, these authors reported for the first time in *C. raciborskii* the presence of mcy A and mcy E, two segments of the MC synthetase mcy cluster in the strain Cyl-BM-07. The strain *C. raciborskii* (MG) that was confirmed not producing cylindrospermopsin present only the PKS gene (Fathalli *et al.* 2010). Schembri *et al.* (2001) confirmed that both the PS and the PKS genes were associated with the ability to produce cylindrospermopsins.

PHYLOGENY

As previously reported in the literature, the research performed on the phylogeny of Tunisian cyanobacteria strains confirmed that the 16S rRNA, which is commonly used to distinguish broad phylogenetic relationships among cyanobacteria because of availability of many sequences of this gene for different species of cyanobacteria, may not fully discriminate closely related species even when they are morphologically distinct. In fact, the phylogenic tree based on 16S rDNA sequences showed that three morphospecies of *Microcystis*, isolated from Lebna reservoir and assigned to *Microcystis aeruginosa* and *Microcystis wesenbergii*, are indistinguishable from each other, from the reference strain PCC 7806 and from many other known *Microcystis* species and, therefore, this tree did not necessarily correlate to the distinctions between morphospecies (El Herry *et al.* 2008b). To elucidate the phylogenetic relationships of these strains, El Herry *et al.* (2008b) used the PCR amplification and restriction fragment length polymorphism (RFLP) analysis of the 16S-23S rRNA spacer region (ITS) of these three morphospecies of *Microcystis,* that provided a similar pattern type for the whole strains. However, it was shown that this region is a good tool to discriminate bacteria strains at the species level and interspecific (Jensen *et al.* 1993; Neilan 1995). Phylogenetic study based on ITS sequences of *Microcystis aeruginosa* strains isolated from five Tunisian waters bodies with several other morphospecies listed in the NCBI database, revealed the existence of two clusters. The first one was formed by most of the Tunisian isolates that were

Table 4 | Toxicity assessment of cyanobacteria in Tunisian freshwater

Cyanobacteria	Origins	Samples	Tested genes number[a]	Expressed genes (PCR+ sequencing)	Toxins	Methods	references
Oscillatoria spp. + *Pseudoanabaena spp.*	Hjar	natural			MC-LR, MC-RR, MC-YR	HPLC/MS/MS	El Herry *et al.* (2007)
Microcystis aeruginosa	Lebna	natural	*3*	*mcyA, -B and -C*	MC	PP2A	El Herry *et al.* (2008b)
Microcystis aeruginosa	Lebna	Culture	*11*	*mcyB,-C,-D,-E* and-*G*	No toxins	MALDI-TOFMS	Fathalli *et al.* (2011a), Fathalli (2012)
Microcystis aeruginosa	Bir M'cherga	Culture	11	*mcy B* and-*G*	No toxins	MALDI-TOFMS	Fathalli *et al.* (2011a), Fathalli, (2012)
Microcystis aeruginosa	Hjar	Culture	11	*mcyA,-B,-C,-D,-E* and-*G*	MC-LR, MC-FR, MC-RR MC-WR, Demethyl-MC-LR	MALDI-TOFMS	Fathalli *et al.* (2011a), Fathalli (2012)
Microcystis aeruginosa	Nebhana	Culture	11	*mcyA,-B,-C,-D,-E* and-*G*	MC-LR, MC-YR	MALDI-TOFMS	Fathalli *et al.* (2011a), Fathalli (2012)
Microcystis aeruginosa	Joumine	Culture	11	*mcyA,-B,-C,-D,-E* and-*G*	MC-LR, MC-YR, Demethyl-MC-LR, Demethyl-MC-YR	MALDI-TOFMS	Fathalli *et al.* (2011a), Fathalli (2012)
Microcystis aeruginosa	Séjnène	Culture	11	*mcyA,-B,-C,-D,-E* and-*G*	MC-LR, MC-RR, MC-YR, Demethyl-MC-LR, Demethyl-MC-RR, Demethyl-MC-YR	MALDI-TOFMS	Fathalli *et al.* (2011a), Fathalli (2012)
Microcystis wesenbergii.	Lebna	Natural	3	*mcyA, -B* and *-C*	MC	PP2A	El Herry *et al.* (2008b)
Oscillatoria tenuis	Lebna	Natural	3	*mcyA, -B* and *-C*	MC	PP2A	El Herry *et al.* (2008a)
Planktothrix agardhii	Joumine	Culture	11	*mcyA*	No toxins	MALDI-TOFMS	Fathalli *et al.* (2011a), Fathalli (2012)
Leptolyngbya sp.	Nebhana	Culture	11	*mcyA*	No toxins	MALDI-TOFMS	Fathalli *et al.* (2011a), Fathalli (2012)
Cylindrospermopsis raciborskii	Bir M'cherga	Culture	11	PKS (MG strain) *mcyA* and *mcyE* (CYL-BM-07 strain)	No toxins	Elisa , HPLC MALDI-TOFMS	Fathalli *et al.* (2010, 2011b): Fathalli (2012)

[a]Number of genes tested; 3: *mcyA, -B* and *-C* (NMT domain of the MC synthetase gene)
11: *mcyA,-B,-C,-D,-E,-G* (MC synthetase gene cluster); AMT (hepatotoxins); GAMT, PS, PKS (cylindrospermopsin synthase); sxt1 (STXs).

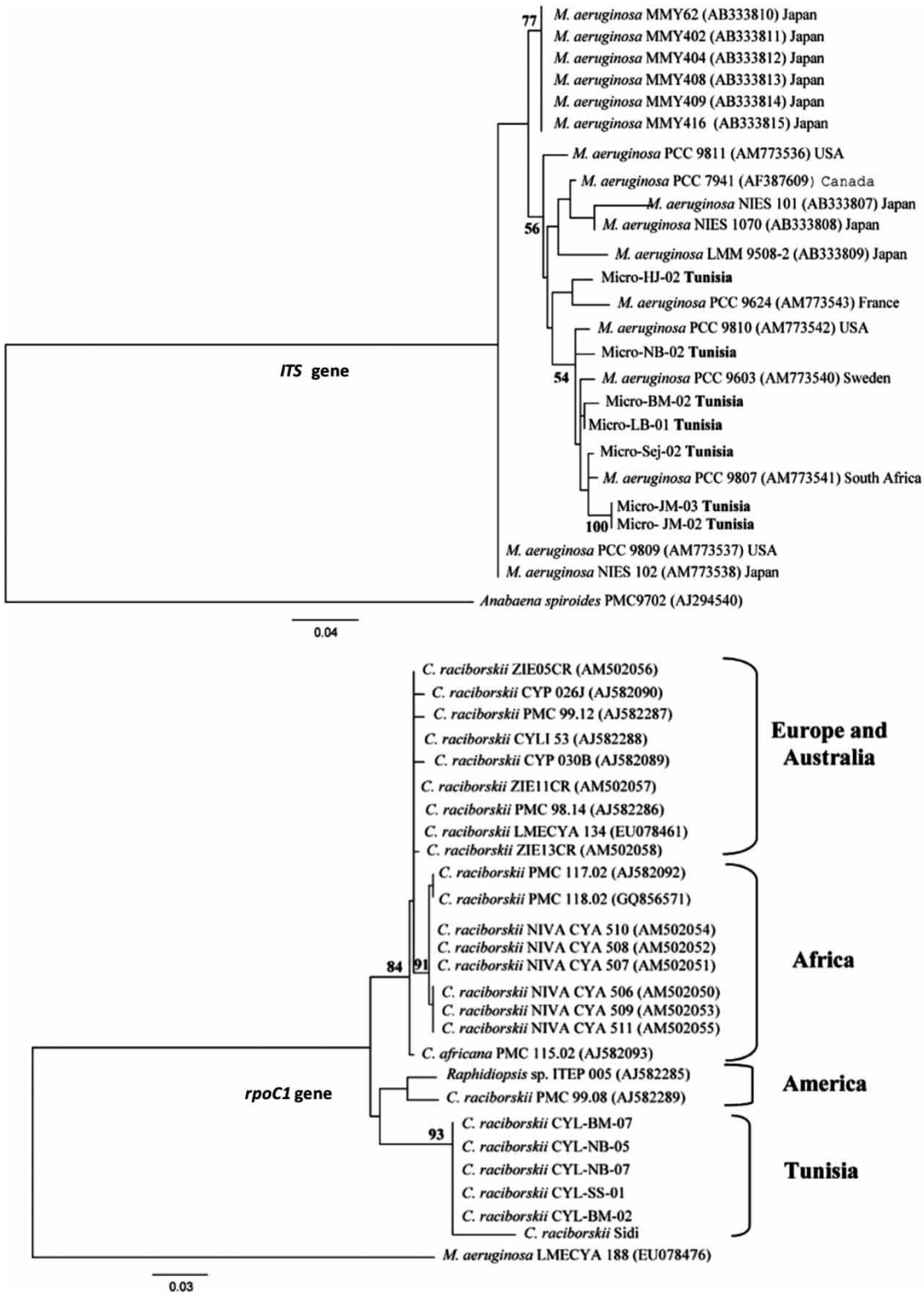

Figure 4 | Neighbour-joining phylogenetic trees based on ITS gene for *Microcystis aeruginosa* species and based on *rpoC1* gene for *Cylindrospermopsis raciborskii* species. Bootstrap values are placed at each branch point (Fathalli *et al*. 2011a).

highly similar to *M. aeruginosa* from the continents of Africa, Europe and America. The second one was composed of one toxic *Microcystis* strain that also clustered with European strains (Fathalli *et al.* 2011a). None of the Tunisian *M. aeruginosa* strains were similar to those found in Asia. No clustering was observed between the toxic and non-toxic *Microcystis* strains; although a well defined sub-group was formed by two non-toxic Tunisian *Microcystis* strains (Figure 4) (Fathalli *et al.* 2011a). Otsuka *et al.* (1999) reported that the same cluster included all the *Microcystis novacekii* and *M. ichthyoblabe* strains and most *M. aeruginosa* strains. These authors also noted that MC-synthesizing and non-producing genotypes were closely related. Tillett *et al.* (2001) confirmed that the analysis of the ITS region was not helpful in distinguishing between MC-producing and non-producing.

Tunisian isolates of *C. raciborskii* were highly similar to each other and they formed a distinct cluster based on *rpoC1* sequences, which was separate from other African strains collected in Senegal and Uganda. In fact, the phylogenetic tree based on rpoC1 sequences separated our species from American, African and European/Australian clusters (Figure 4) (Fathalli *et al.* 2011a). The *rpoC1* gene was reported to be more discriminatory at the species level than the 16S rRNA, for this species (Wilson *et al.* 2000). This different clustering of the African strains demonstrates that the population structure in this continent is somewhat heterogeneous, supporting the uniqueness of the Tunisian isolates relative to *C. raciborskii* strains from other geographical locations (Fathalli *et al.* 2011b). Moreira *et al.* (2011) showed, based on a concatenated fragment of 2.9 Kb encompassing the four genetic markers 16S rRNA, ITS longer spacer, ITS shorter spacer and *rpoC1* sequences, that the Tunisian *C. raciborskii* strains were clustered with the American strain. In fact, this study revealed that the *C. raciborskii* strains grouped in to three well-supported distinct clusters: (i) European, (ii) African (Tunisian)/American (Brazilian) and (iii) Asian/Australian. This method provided a better phylogeographical distinction of the strains, with higher statistical significance than if considering the information of each gene alone. In fact, several authors attribute the recent dispersion of *C. raciborskii* to migration of water birds, like gulls, that carry these organisms in their intestinal tract or even owing to the importation of items from tropical countries that bring resistant cyanobacterial cells that can later grow when suitable conditions are found (Manti *et al.* 2005; Vasconcelos 2006).

CONCLUSION

In Tunisia, the increase in cyanobacterial occurrence, observed in some water bodies, poses a potential risk to the environment and public health. *Microcystis*, one of the most widely distributed toxic bloom-forming cyanobacteria genera, was represented mainly by *Microcytis aeruginosa* recorded in different water bodies. The invasive potentially toxic cyanobacterium *Cylindrospermopsis raciborskii* was observed in the semi-arid to arid areas. The harmful genus *Planktothrix* was represented in Tunisian freshwater by the green-pigmented specie *P. agardhii*. The filamentous cyanobacteria dominance is increasingly reported in Tunisia in eutrophic water bodies. These cyanobacteria which synthesize secondary metabolites responsible for the deterioration of water quality and their uses did not form real blooms at all prospected water bodies, except for el Hjar and Lebna reservoirs. Thus, the question remains, 'How long will these environments still be safe?' knowing that neighboring countries (Morocco and Algeria) currently are faced with the complexity of this phenomenon.

REFERENCES

Abdel-Rahman, S., El-Ayouty, Y. M. & Kamael, H. A. 1993 Characterization of heptapeptide toxins extracted from *Microcystis* aeruginosa (Egyptian isolate): comparison with some synthesized analogs. *Int. J. Pept. Protein. Res.* **41**, 1–7.

Aboal, M. & Puig, M. A. 2005 Intracellular and dissolved microcystin in reservoirs of the river Segura basin, Murcia, SE Spain. *Toxicon* **45**, 509–518.

Albay, M., Akcaalan, R., Aykulu, G., Tufekci, H., Beattie, K. A. & Codd, G. A. 2003 Occurrence of toxic cyanobacteria before and after copper sulphate treatment in a water reservoir, Istanbul, Turkey. *Arch. Hydrobiol. Suppl. Algol. Stud.* **109**, 67–78.

Albay, M., Matthiensen, A. & Codd, G. A. 2005 Occurrence of toxic blue-green algae in the Kucukcekmece Lagoon (Istanbul, Turkey). *Environ. Toxicol.* **20**, 277–284.

Alster, A., Kaplan-Levy, R. N., Sukenik, A. & Zohary, T. 2010 Morphology and phylogeny of a non-toxic invasive *Cylindrospermopsis raciborskii* from a Mediterranean Lake. *Hydrobiologia* **639**, 115–128.

Baker, J. A., Neilan, B. A., Entsch, B. & McKay, D. B. 2001 Identification of cyanobacteria and their toxigenicity in

environmental samples by rapid molecular analysis. *Environ. Toxicol.* **16**, 472–482.

Ballot, A., Krienitz, L., Kotut, K., Wiegand, W. & Pflugmacher, S. 2005 Cyanobacteria and cyanobacterial toxins in the alkaline crater lakes Sonachiand Simbi, Kenya. *Harmful Algae* **4**, 139–150.

Belay, A. 1997 Mass culture of *Spirulina* outdoors – the Earth-rise Farms experience. In: *Spirulina platensis (Arthrospira): Physiology, Cell-Biology and Biotechnology* (A. Vonshak, ed.). Taylor & Francis, London.

Ben Boubaker, H., Benzarti, Z. & Hénia, L. 2003 Les ressources en eaux de la Tunisie: contraintes du climat et pression anthropique. In: *Eau et environnement: Tunisie et milieux méditérranéens* (P. Arnould & M. Hotyat, eds). ENS, Lyon.

Ben Mammou, A. & Louati, M. H. 2007 Évolution temporelle de l'envasement des retenues de barrages de Tunisie. *Revue des Sciences de l'Eau* **20**, 201–211.

Ben Rejeb Jenhani, A. 1990 *Etude qualitative, quantitative et structurale des peuplements phytoplanctoniques des eaux du barrage de sidi salem*. Rapports techniques, Projet Tuniso-Allemant de Pêche continentale, Tunis, Tunisia.

Ben Rejeb Jenhani, A., Bouaicha, N., El Herry, S., Fathalli, A., Zekri, I., Haj Zekri, S., Limam, A., Alouini, S. & Romdhane, M. S. 2006 Les cyanobactéries et leurs potentialities toxiques dans les retenues des retenues des barrages du Nord de la Tunisie. *Archives de l'Institut Pasteur de Tunis* **83**, 1–4.

Ben Rejeb Jenhani, A., Fathalli, A., Moreira, C., Antunes, A., Romdhane, M. S. & Vasconcelos, V. 2010 Molecular techniques for the toxicological assessment of cyanobacterial blooms in Tunisian freshwaters. In *Proceedings of the 8th International Conference on Toxic Cyanobacteria ICTC8*. Istanbul, Official Program and Abstract book, Turkey.

Benzarti, Z. 2003 La pluviométrie en Tunisie: Analyse des années très pluvieuse. In: *Eau et environnement: Tunisie et milieux méditérranéens* (P. Arnould & M. Hotyat, eds). ENS, Lyon.

Bergaoui, M. & Louati, M. H. 2010 Drought effects on reservoirs inflows in Tunisia: case of Lakhmess and Siliana reservoirs. In: *Options Méditerranéennes A, Economics of drought and drought preparedness in a climate change context. Vol*. **95**, pp. 75–78.

Berger, C. & Sweers, H. E. 1988 The Ijsselmeer and its phytoplankton with special attention to the suitability of the lake as a habitat for *Oscillatoria* agardhii Gom. *J. Plankton. Res.* **10**, 579–599.

Bernard, C., Harvey, M., Briand, J. F., Bire, R., Fontaine, J. J. & Krys, S. 2003 Liver damage caused by a French *Cylindrospermopsis raciborskii* strain: comparison with diverse *C. raciborskii* toxic strains. *Environ. Toxicol.* **18**, 176–186.

Bernard, C., Froscio, S., Campbell, R., Monis, P., Humpage, A. & Fabbro, L. 2011 Novel toxic effects associated with a tropical *Limnothrix*/Geitlerinema-like cyanobacterium. *Environ. Toxicol.* **6**, 260–270.

Bouaïcha, N. & Nasri, A. B. 2004 First report of cyanobacterium *Cylindrospermopsis raciborskii* from Algerian freshwaters. *Environ. Toxicol.* **19**, 541–543.

Bourke, A. T. C., Hawes, R. B., Nielson, A. & Stallman, N. D. 1983 An outbreak of the Palm Island Dystery disease possibly caused by algal intoxication. *Toxicon* **21**, 45–48.

Briand, J. F., Robiliot, C., Quiblier-Llobéras, C., Humbert, J. F., Couté, A. & Bernard, C. 2002 Environmental context of *Cylindrospermopsis raciborskii* (cyanobacteria) blooms in a shallow pond in France. *Water Res.* **36**, 3183–3192.

Carmichael, W. W. 1992 Cyanobacteria secondary metabolites – the cyanotoxins. *J. Appl. Bacteriol.* **2**, 445–459.

Carmichael, W. W. 1995 Toxic *Microcystis* and the environment. In: *Toxic Microcystis* (M. Watanabe, K. Harada, W. Carmichael & H. Fujiki, eds). CRC Press, Boca Raton, Florida.

Carmichael, W. W. 2001 Health effects of toxin-producing cyanobacteria: 'The CyanoHABs'. *Hum. Ecol. Risk Assess.* **7**, 1393–1407.

Carmichael, W. W. & Falconer, I. R. 1993 Diseases related to freshwater blue-green algal toxins and control measures. In: *Algal Toxins in Seafood and Drinking Water* (I. Falconer, ed.). Academic Press, London.

Cherif, A. 2003 Le problème de l'eau en Tunisie nord-orientale: besoin ressource locales et transferts interrégionaux. In: *Eau et environnement: Tunisie et milieux méditérranéens* (P. Arnould & M. Hotyat, eds). ENS Edition, Lyon.

Chorus, I. & Bartram, J. (eds) 1999 *Toxic Cyanobacteria in water. A guide to their public Health consequences, monitoring and management*, WHO & EF&N Spon, London.

Ciferri, O. 1983 *Spirulina*, the edible micro-organism. *Microbiol. Rev.* **47**, 551–578.

Codd, G. A. 2000 Cyanobacterial toxins, the perception of water quality and the prioritization of eutrophication control. *Ecol. Eng.* **16**, 51–60.

Codd, G. A., Morrison, L. F. & Metcalf, J. S. 2005 Cyanobacterial toxins: risk management for health protection. *Toxicol. Appl. Pharm.* **203**, 264–272.

Dauta, A. & Feuillade, J. 1995 Croissance et dynamique des populations algales. In: *Limnologie générale*, *Collection Ecologie* (R. Pourriot & M. Meybeck, eds). Masson, Paris/Milan/Barcelona, pp. 328–350.

Dittmann, E., Neilan, B. A., Erhard, M., Von Dohren, H. & Borner, T. 1997 Insertional mutagenesis of a peptide synthetase gene that is responsible for hepatotoxin production in *Microcystis aeruginosa* PCC7806. *Mol. Microbiol.* **26**, 779–784.

Dittmann, E., Neilan, B. A. & Börner, T. 2001 Molecular biology of peptide and polyketide biosynthesis in cyanobacteria. *Appl. Microbiol. Biotechnol.* **57**, 467–473.

Dokulil, M. T. & Teubner, K. 2000 Cyanobacterial dominance in lakes. *Hydrobiologia* **438**, 1–12.

Douma, M., Ouahid, Y., del Campo, F. F., Loudiki, M., Mouhri, K. & Oudra, B. 2010 Identification and quantification of cyanobacterial toxins (microcystins) in two Moroccan drinking-water reservoirs (Mansour Eddahbi, Almassira). *Environ. Monit. Assess.* **160**, 439–450.

El Herry, S., Bouaïcha, N., Jenhani Ben Rejeb, A. & Romdhane, M. S. 2007 First observation of *Microcystins* in Tunisian inland waters: a threat to river mouths and lagoon ecosystems. *Transit. Waters Bull.* **2**, 73–82.

El Herry, S., Fathalli, A., Jenhani Ben Rejeb, A. & Bouaïcha, N. 2008a Seasonal occurrence and toxicity of *Microcystis spp.*

and *Oscillatoria* tenuis in the Lebna Dam, Tunisia. *Water Res.* **42**, 1263–1273.

El Herry, S., Nasri, H. & Bouaïcha, N. 2008b Morphological and phylogenetic analysis of colonies of *Microcystis* morphospecies isolated from the Lebna Dam, Tunisia. *Afr. J. Microbiol. Res.* **2**, 340–348.

El Herry, S., Nasri, H. & Bouaïcha, N. 2009 Morphological characteristics and phylogenetic analyses of unusual morphospecies of *Microcystis* novacekii forming bloom in the Cheffia Dam (Algeria). *J. Limnol.* **68**, 242–250.

Fastner, J., Heinze, R., Humpage, A. R., Mischke, U. K., Eaglesham, G. & Chorus, I. 2003 Cylindrospermopsin occurrence in two German lakes and preliminary assessment of toxicity and toxin production of *Cylindrospermopsis raciborskii* (cyanobacteria) isolates. *Toxicon* **42**, 313–321.

Fathalli, A. 2012 *Caractérisation moléculaire et étude du potentiel toxique des souches de cyanobactéries isolées à partir des retenues de barrages de la Tunisie: Cas de la retenue du barrage Bir M'cherga*. PhD thesis, University of Carthage, Tunisia.

Fathalli, A., Ben Rejeb Jenhani, A., Romdhane, M. S. & Bouaïcha, N. 2006 Contribution à la caractérisation phytoplanctonique et écotoxicologique des eaux de la retenue de barrage Kasseb. *Water, Waste and Environ. Res.* **6**, 33–42.

Fathalli, A., Jenhani, A., Saker, M., Moreira, C., Romdhane, M. S. & Vasconcelos, V. M. 2010 First observation of the toxic and invasive cyanobacterium species *Cylindrospermopsis raciborskii* in Tunisian freshwaters. Toxicity assessment and molecular characterization. *Fresen. Environ. Bull.* **19**, 1074–1083.

Fathalli, A., Ben Rejeb Jenhani, A., Moreira, C., Welker, M., Romdhane, M., Antunes, A. & Vasconcelos, V. 2011a Molecular and phylogenetic characterization of potentially toxic cyanobacteria in Tunisian freshwaters. *Sys. Appl. Microbiol.* **34**, 303–310.

Fathalli, A., Ben Rejeb Jenhani, A., Moreira, C., Azevedo, J., Welker, M., Romdhane, M., Antunes, A. & Vasconcelos, V. 2011b Genetic variability of the invasive cyanobacteria *Cylindrospermopsis raciborskii* from Bir M'cherga reservoir (Tunisia). *Arch. Microbiol.* **193**, 595–604.

Fergusson, K. M. & Saint, C. P. 2003 Multiplex PCR Assay for *Cylindrospermopsis raciborskii* and cylindrospermopsin-producing cyanobacteria. *Environ. Toxicol.* **18**, 120–125.

Francis, G. 1878 Poisonous Australian Lake. *Nature* **18**, 11–12.

Haande, S., Rohrlack, T., Ballot, A., Røberg, K., Skulberg, R., Beck, M. & Wiedner, C. 2008 Genetic characterisation of *Cylindrospermopsis raciborskii* (Nostocales, cyanobacteria) isolates from Africa and Europe. *Harmful Algae* **7**, 692–701.

Hawkins, P. R., Runnegar, M. T. C., Jackson, A. R. B. & Falconer, I. R. 1985 Severe hepatotoxicity caused by the tropical cyanobacterium (blue-green alga) *Cylindrospermopsis raciborskii* (Woloszynska) Seenaya and Subba Raju isolated from a domestic water supply reservoir. *Appl. Environ. Microbiol.* **50**, 1292–1295.

Hawkins, P. R., Chandrasena, N. R., Jones, G. J., Humpage, A. R. & Falconer, I. R. 1997 Isolation and toxicity of *Cylindrospermopsis raciborskii* from an ornamental lake. *Toxicon* **35**, 341–346.

Heisler, J. P., Gilbert, J., Burkholder, J., Anderson, D., Cochlan, W., Dennison, W., Dortch, Q., Gobler, C. J., Heil, C., Humphries, E., Lewitus, A., Magnien, R., Marshall, H., Sellner, K., Stockwell, D., Stoecker, D. & Suddleson, M. 2008 Eutrophication and harmful algal blooms: a scientific consensus. *Harmful Algae* **8**, 3–13.

Hoagland, P., Anderson, D. M., Kaoru, Y. & White, A. W. 2002 The economic effects of harmful algal blooms in the United States: estimates, assessment issues and information needs. *Estuaries* **25**, 819–837.

Hudnell, K. H. 2008 Cyanobacterial harmful algal blooms: state of the science and research needs. In: *Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs* (K. H. Hudnell, ed.). Advances in Experimental Medicine and Biology. Springer, New York.

Humpage, A., Falconer, I., Bernard, C., Froscio, S. & Fabbro, L. 2012 Toxicity of the cyanobacterium *Limnothrix* AC0243 to male Balb/c mice. *Water Research* **46**, 1576–1583.

INSTM 2006 *Ecotoxicologie et Suivi de la Santé des Animaux Marins et des Eaux Continentales*. Institut National des Sciences et Technologies de la Mer (INSTM), Tunisia.

Janse van Vuuren, S. & Kriel, G. P. 2008 *Cylindrospermopsis raciborskii*, a toxic invasive cyanobacterium in South African freshwaters. *Afr. J. Aquat. Sci.* 33, 17–26.

Jensen, M., Webster, J. & Straus, N. 1993 Rapid identification of bacteria on the basis of polymerase chain reaction amplified ribosomal DNA spacer polymorphisms. *Appl. Environ. Microb.* **59**, 502–539.

Jochimsen, E. M., Carmichael, W. W., An, J., Cardo, D. M., Cookson, S. T., Áholmes, C. E. M., Antunes, M. B., Melo, D. A., Lyra, T. M., Spinelli, V. T., Azevedo, S. M. F. O. & Jarvis, W. R. 1998 Liver failure and death following exposure to microcystin toxins at a dialysis center in Brazil. *New Eng. J. Med.* **338**, 873–878.

Kellmann, R. & Neilan, B. A. 2007 Biochemical characterization of paralytic shellfish toxin biosynthesis in vitro. *J. Phycol.* **43**, 497–508.

Kellmann, R., Mihali, T. K., Jeon, Y. J., Pickford, R., Pomati, F. & Neilan, B. A. 2008 Biosynthetic intermediate analysis and functional homology reveal a saxitoxin gene cluster in cyanobacteria. *Appl. Environ. Microbiol.* **74**, 4044–4053.

Komàrek, J. & Anagnostidis, K. 1999 Cyanoprokaryota1, Teil: Chroococcales. In: *Sußwasser floravon Mitteleuropa Band19/1* (H. Ettl, G. Gartner, H. Heynig & D. Mollenhauer, eds). Gustav Fischer Verlag, Stuttgart, Germany, pp. 9–999.

Kosten, S., Huszar, V., Bécares, E., Costa, L., vanDonk, E., Hansson, L.-A., Jeppessn, E., Kruk, C., Lacerot, G., Mazzeo, N., DeMeester, L., Moss, B., Lurling, M., Nõges, T., Romo, S. & Scheffer, M. 2012 Warmer climate boosts cyanobacterial dominance in shallow lakes. *Global. Change. Biol.* **18**, 118–126.

Kümmerlin, R. 1991 Abschlussbericht zum Project 'Protection et exploitation du barrage Sidi Salem.'. MS: 1–12 (*Phytoplancton-retenue du barrage Sidi Salem*).

Kurmayer, R., Christiansen, G., Gumpenberger, M. & Fastner, J. 2005 Genetic identification of microcystin ecotypes in toxic cyanobacteria of the genus *Planktothrix*. *Microbiology* **151**, 1525–1533.

Lagos, N., Onodera, H., Zagatto, P. A., Andrinolo, D., Azevedo, S. M. F. Q. & Oshima, Y. 1999 The first evidence of paralytic shellfish toxins in the freshwater cyanobacterium *Cylindrospermopsis raciborskii*, isolated from Brazil. *Toxicon* **37**, 1359–1373.

Lee, S. J., Jang, M. H., Kim, H. S., Yoon, B. D. & Oh, H. M. 2000 Variation of microcystin content of *Microcystis aeruginosa* relative to medium N:P ratio and growth stage. *J. Appl. Microbiol.* **89**, 323–329.

Li, R., Carmichael, W. W. & Brittain, S. 2001 First report of the cyanotoxins cylindrospermopsin and deoxycylindrospermopsin from *Raphidiopsis curvata* (cyanobacteria). *J. Phycol.* **37**, 1121–1126.

Manti, G., Mattei, D. & Messineo, V. 2005 First report of *Cylindrospermopsis raciborskii* in Italy. *Harmful Algal News* **28**, 8–9.

Mei Li, D. & Zao Qi, Y. 1997 Spirulina industry in China: present status and future prospects. *J. Appl. Phycol.* 9, 25–28.

Messineo, V., Melchiorre, S., DiCorcia, A., Gallo, P. & Bruno, M. 2010 Seasonal succession of *Cylindrospermopsis raciborskii* and Aphanizomenon ovalisporum blooms with cylindrospermopsin occurrence in theVolcanic Lake Albano, Central Italy. *Environ. Toxicol.* **I25**, 18–27.

Mohamed, Z. A. 2007 First report of toxic *Cylindrospermopsis raciborskii* and *Raphidiopsis mediterranea* (Cyanoprokaryota) in Egyptian freshwaters. *FEMS Microbiol. Ecol.* **59**, 749–761.

Moreira, C., Fathalli, A., Vasconcelos, V. & Antunes, A. 2011 Genetic diversity and structure of the invasive toxic cyanobacterium *Cylindrospermopsis raciborskii*. *Curr. Microbiol.* **62**, 1590–1595.

Mouelhi, S. 2000 *Etude écologique de la retenue de Sidi Salem: Aspects physico-chimiques des eaux et dynamique des peuplements zooplanctoniques*. PhD thesis, University of Tunis II, Tunisia.

Moustaka-Gouni, M., Vardaka, E. & Tryfon, E. 2007 Phytoplankton species succession in a shallow Mediterranean lake (L. Kastoria, Greece): steady-state dominance of *Limnothrix redekei*, *Microcystis aeruginosa* and *Cylindrospermopsis raciborskii*. *Hydrobiologia* **575**, 129–140.

Nasri, A. B., Bouaïcha, N. & Fastner, J. 2004 First report of a microcystin-containing bloom of the cyanobacteria *Microcystis* spp. In Lake Oubeira, eastern Algeria. *Arch. Environ. Contam. Toxicol.* **46**, 197–202.

Nasri, H., El Herry, S. & Bouaïcha, N. 2008 First reported case of turtle deaths during a toxic *Microcystis* spp. bloom in Lake Oubeira, Algeria. *Ecotoxicol. Environ. Safety* **71**, 535–544.

Neilan, B. A. 1995 Identification and phylogenetic analysis of toxigenic cyanobacteria by multiplex randomly amplified polymorphic DNA PCR. *Appl. Environ. Microbiol.* **61**, 2286–2291.

Nishizawa, T., Ueda, A., Asayama, M., Fujii, K., Harada, K., Ochi, K. & Shirai, M. 2000 Polyketide synthase gene coupled to the peptide synthetase module involved in the biosynthesis of the cyclic heptapeptide microcystin. *J. Biochem. (Tokyo)* **127**, 779–789.

Otsuka, S., Suda, S., Li, R., Watanabe, M., Oyaizu, H., Matsumoto, S. & Watanabe, M. M. 1999 Phylogenetic relationships between toxic and non-toxic strains of the genus *Microcystis* based on 16S to 23S internal transcribed spacer sequence. *FEMS Microbiol. Lett.* **172**, 15–21.

Ouahid, Y., Pérez-Silva, G. & del Campo, F. F. 2005 Identification of potentially toxic environmental *Microcystis* by individual and multiple PCR amplification of specific microcystin synthetase gene regions. *Environ. Toxicol.* **20**, 235–242.

Oudra, B., Loudiki, M., Sbiyyaa, B., Martins, R., Vasconcelos, V. & Namikoshi, M. 2001 Isolation, characterization and quantification of microcystins (heptapeptides hepatotoxins) in *Microcystis aeruginosa* dominated bloom of Lalla Takerkoust lake reservoir (Morocco). *Toxicon* **39**, 1375–1381.

Oudra, B., Loudiki, M., Sabour, B., Sbiyyaa, B. & Vasconcelos, V. 2002 Etude des blooms toxiques à cyanobactéries dans trois lacs réservoirs du Maroc: résultats préliminaires. *Revue des Sciences de l'Eau* **15**, 303–313.

Ouellette, A. J. A. & Wilhelm, S. W. 2003 Toxic cyanobacteria: the evolving molecular toolbox. *Front Ecol. Environ.* **1**, 359–366.

Padisák, J., 1997 *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya et Subba Raju, an expanding highly adaptive blue-green algal species: worldwide distribution and review of its ecology. *Arch. Hydrobiol. Suppl.* **107**, 563–593.

Paerl, H. W. 1988 Nuisance phytoplankton blooms in coastal, estuarine, and inland waters. *Limnol. Oceanogr.* **33**, 823–847.

Paerl, H. W. 2008 Nutrient and other environmental controls of harmful cyanobacterial blooms along the freshwater-marine continuum. *Adv. Exp. Med. Biol.* **619**, 216–241.

Paerl, H. W. & Huisman, J. 2008 Blooms like it hot. *Science* **320**, 57–58.

Paerl, H. W. & Paul, V. J. 2012 Climate change: links to global expansion of harmful cyanobacteria. *Water Res.* **46**, 1349–1363.

Paul, V. J. 2008 Global warming and cyanobacterial harmful algal blooms. In: *Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs* (H. K. Hudnell, ed.). Advances in Experimental Medicine and Biology. Springer, New York.

Ressom, R., Soong, F. S., Fitzgerald, J., Turczynowicz, L., El Saadi, O., Roder, D., Maynard, T. & Falconer, I. R. 1994 *Health effects of toxic cyanobacteria (blue-green algae)*. Australian Government Publishing Service, Canberra, Australia.

Reynolds, C. S., Huszar, V. L. M., Kruk, J., Naselli-Flores, L. & Melo, S. 2002 Towards a functional classification on the freshwater phytoplankton. *J. Plankton. Res.* **24**, 417–428.

Romdhane, M. S., Ben Rejeb Jenhani, A., Limam, A., Fathalli, A., El Herry, S., Zekri, I., Belhaj Zekri, S., Alouni, S. & Louati, M. H. 2006 Inventaire et distribution de la microflore des eaux continentales du Nord de la Tunisie. In: *Proceedings of the 8ème Conférence Internationale des Limnologues d'Expression Française CILEF*, Hammamet, Tunisia.

Romo, S. & Miracle, M. R. 1994 Population dynamics and ecology of subdominant phytoplankton species in a shallow hypertrophic lake (Abulifera of Valencia, Spain). *Hydrobiologia* **273**, 37–56.

Sabour, B., Loudiki, M., Oudra, B., Vasconcelos, V., Martins, R., Oubraim, S. & Fawzi, B. 2002 Toxicity and toxinology of *Microcystis ichtyoblabe* waterbloom occurred in the Oued Mellah Lake (Morocco). *Environ. Toxicol.* **17**, 24–31.

Saker, M. L., Thomas, A. D. & Norton, J. H. 1999 Cattle mortality associated with the toxic cyanobacterium *Cylindrospermopsis raciborskii* in an outback region of north Queensland. *Environ. Toxicol.* **14**, 179–182.

Saker, M. L., Nogueira, I. C. G., Vasconcelos, C. M., Neilan, B. A., Eaglesham, G. K. & Pereira, P. 2003 First report and toxicological assessment of the cyanobacterium *Cylindrospermopsis raciborskii* from Portuguese freshwaters. *Ecotoxicol. Environ. Saf.* **55**, 243–250.

Saqrane, S. & Oudra, B. 2009 CyanoHAB occurrence and water irrigation cyanotoxin contamination: ecological impacts and potential health risks. *Toxins* **1**, 113–122.

Scheffer, M. 1998 *Ecology of shallow lakes*. Chapman & Hall, London.

Schembri, M. A., Neilan, B. A. & Saint, C. P. 2001 Identification of genes implicated in toxin production in the cyanobacterium *Cylindrospermopsis raciborskii*. *Environ. Toxicol.* **16**, 413–421.

Schopf, W. J. 2000 The fossil record: tracing the roots of the cyanobacterial lineage. In: *The Ecology of Cyanobacteria. Their Diversity in Time and Space* (B. A. Whitton & M. Potts, eds). Kluwer Academic Publishers, Dordrecht, The Netherlands.

Sellami, I., Guermazi, W., Hamza, A., Aleya, L. & Ayadi, H. 2010 Seasonal dynamics of zooplankton community in four Mediterranean reservoirs in humid area (Beni Mtir: north of Tunisia) and semi arid area (Lakhmes, Nabhana and Sidi Saâd: center of Tunisia). *J. Therm. Biol.* **35**, 392–400.

Sellami, I., Ben Romdahane, S., Guermazi, W., El Bour, M., Hamza, A., Alaoui Mhamdi, M., Pinel-Alloul, B., Aleya, L. & Ayadi, H. 2012 Seasonal dynamics of plankton communities coupled with environmental factors in a semi arid area: Sidi Saâd reservoir (Center of Tunisia). *African J. Biotechnol.* **11**, 865–877.

Silva, P. & Vasconcelos, V. 2010 Allelopathic effect of *Cylindrospermopsis raciborskii* extracts on the germination and growth of several plant species. *Chem. Ecol.* **26**, 263–271.

Sivonen, K. 1998 Toxins produced by cyanobacteria. In: M. Miraglia, H. Van Egmont, C. Brera & J. Gilbert, ed.). *Mycotoxins and Phycotoxins – Developments in Chemistry, Toxicology and Food Safety*. International Union of Pure and Applied Chemistry (IUPAC), Oxford.

Sivonen, K. & Börner, T. 2008 Bioactive compounds produced by cyanobacteria. In: *Cyanobacteria: Molecular Biology, Genomics and Evolution* (A. Herrero & G. Flores, eds). Caister Academic Press, Norfolk, UK.

Sivonen, K. & Jones, G. 1999 Cyanobacterial toxins. In: *Toxic Cyanobacteria in Water* (I. Chorus & J. Bartram, eds). E&FN Spon, London.

Teneva, I., Dzhambazov, B., Koleva, L., Mladenov, R. & Schirmer, K. 2005 Toxic potential of five freshwater *Phormidium* species (cyanoprokaryota). *Toxicon* **45**, 711–725.

Terao, K., Ohmori, S., Igarshi, K., Ohtani, I., Watanabe, M. F., Harada, K. I., Ito, E. & Watanabe, M. 1994 Electron microscope studies on experimental poisoning in mice induced by cylindrospermopsin isolated from blue-green alga Umezakia natans. *Toxicon* **32**, 833–843.

Tillett, D., Dittmann, E., Erhard, M., von Döhren, H., Börner, T. & Neilan, B. A. 2000 Structural organization of microcystin biosynthesis in *Microcystis aeruginosa* PCC7806: an integrated peptidepolyketide synthetase system. *Chem. Biol.* **7**, 753–764.

Tillett, D., Parker, D. L. & Neilan, B. A. 2001 Detection of toxigenicity by a probe for the microcystin synthetase a gene (mcyA) of the cyanobacterial genus *Microcystis*: comparison of toxicities with 16S rRNA and phycocyanin operon (phycocyanin intergenic spacer) phylogenies. *Appl. Environ. Microbiol.* **67**, 2810–2818.

Torres-Arino, A. & Mora-Heredia, E. 2010 Isolation and characterization of potentially toxic or harmful cyanobacteria from Oaxaca and Chiapas, Mexico. *J. Environ. Sci. Heal. A* **45**, 128–136.

Turki, S. 2002 *Contribution à l'étude bio-écologique des rotifères, cladocères, copépodes des eaux continentales tunisiennes et dynamique saisonnière du zooplancton de la retenue de barrage Bir M'cherga*. PhD thesis, University of Tunis II, Tunisia.

Vardaka, E., Moustaka-Gouni, M., Cook, C. M. & Lanaras, T. 2005 Cyanobacterial blooms and water quality in Greek fresh waters. *J. Appl. Phycol.* **17**, 391–401.

Vasconcelos, V. 2006 Eutrophication, toxic cyanobacteria and cyanotoxins: when ecosystems cry for help. *Limnetica* **25**, 425–432.

Vezie, C., Brient, L., Sivonen, K., Bertru, G., Lefeuvre, J. C. & Salkinoja-Salonen, M. 1998 Variation of microcystin content of cyanobacterial blooms and isolated strains in Lake Grand-Lieu (France). *Microb. Ecol.* **35**, 126–135.

Via-Ordorika, L., Fastner, J., Kurmayer, R., Hisbergues, M., Dittmann, E., Komarek, J., Erhard, M. & Chorus, I. 2004 Distribution of microcystin-producing and non-microcystin-producing *Microcystis sp.* in European freshwater bodies: detection of microcystins and microcystin genes in individual colonies. *System. Appl. Microbiol.* **27**, 592–602.

Vonshak, A. 1997 *Spirulina platensis (Arthrospira): Physiology, Cell-Biology and Biotechnology*. Taylor & Francis, London.

Whitton, B. A. & Potts, M. 2000 *The Ecology of Cyanobacteria. Their Diversity in Time and Space*. Kluwer Academic Publishers, Dordrecht, The Netherlands.

WHO 1998 *Cyanobacterial toxins: Microcystin-LR in Drinking-water. Background document for development of WHO Guidelines for Drinking-water Quality*, 2nd edn. Addendum to Vol. 2 *Health criteria and other supporting information*. World Health Organization, Geneva.

Wilson, K. M., Schembri, M. A., Baker, P. D. & Saint, C. P. 2000 Molecular characterisation of the toxic cyanobacterium *Cylindrospermopsis raciborskii* and design of a species-specific PCR. *Appl. Environ. Microbiol.* **66**, 332–338.

First received 28 August 2014; accepted in revised form 22 January 2015. Available online 5 March 2015