On the occurrence and toxicity of *Cylindrospermopsis raciborskii* in Poland

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Abstract: *Cylindrospermopsis raciborskii* which belongs to the order of Nostocales has continuously been at the centre of interest of various research groups owing to its great ecological plasticity, wide distribution and potential to produce different metabolites known to be harmful for humans and animals. Over recent decades, Polish strains of *C. raciborskii* have also been studied with regard to these issues. The present paper is a brief review of the present state of knowledge respecting the occurrence and toxicity of this species with emphasis on Polish strains, and indicates potential directions for future research.

Key words: cyanobacteria; *Cylindrospermopsis raciborskii*; cyanotoxins; toxicity; Polish lakes

Introduction

*Cylindrospermopsis raciborskii* (Woloszynska 1912) Seenayya et Subba Raju (1972) is a freshwater, planktonic filamentous and nitrogen-fixing cyanobacteria belonging to the Nostocales order (Antunes et al. 2015). It is undoubtedly one of the most widely studied nostocaleans for a number of reasons: (i) its near global distribution encompassing Europe, Asia, North and South America, Africa, Australia and New Zealand, and spanning different climate conditions (Rzymski and Poniedziałek 2014); (ii) its potential to produce various metabolites recognized as toxic to humans and animals that, *inter alia*, include: cylindrospermopsin (CYN), saxitoxins (STXs) and polymethoxy-1-alkenes (PMA) (Ohtani et al. 1992; Jaja-Chimedza et al. 2012; Hoff-Risseti et al. 2013); (iii) the toxicity of certain strains linked to the potential production of hitherto unknown compound(s) (e.g. see Acs et al. 2013; Smutná et al. 2016; Rzymski et al. 2017); (iv) the geographical diversification of toxin production raising questions as to the evolutionary/environmental reasons behind this phenomenon (Gugger et al. 2005; Cirés et al. 2014; Rzymski and Poniedziałek 2014); (v) no scientific consensus as regards the ecological role of produced metabolites, although some hypotheses, e.g. use in allelopathic interactions have been put forward (Holland and Kinnear 2013; Rzymski et al. 2014); (vi) phenotypic plasticity in response to key environmental factors such as temperature, light, nutrient availability (Bonilla et al. 2012); (vii) the occurrence of ecotypes differing in environmental tolerance (Chonudomkul et al. 2004; Bonilla et al. 2016); (viii) the potential for future expansion to new environments and an invasive nature owing to reasons given in the three previous points (Padisák and Reynolds 1998; Sinha et al. 2012; Engström-Öst et al. 2015).

*C. raciborskii* is a potent bloom forming species, usually found in eutrophic lakes regardless of geographical region (Kokociński et al. 2013; Soares et al. 2013), although its occurrence in low-land rivers has also been reported (Karadžić et al. 2013). It can easily be distinguished from other cyanobacteria under a light microscope by means of morphological features. Its trichomes are solitary and usually straight but coil forms are also known to occur. Cells are cylindrical or barrel shaped, longer than wide, slightly to distinctly constricted at the cross-walls within the trichome. The terminal position of an elongated drop-like shape heterocyst is a characteristic feature of this species. During unfavorable environmental conditions akinetes are
formed. Usually two or three elongated, oval akinetes are located in a row near the ends of trichomes, separated from the heterocyte by a single vegetative cell. The typical morphological forms of *C. raciborskii* which can be observed in Polish lakes are presented in Figure 1.

A comprehensive, multi-approach evaluation of the distribution, frequency of occurrence and potential toxicity of *C. raciborskii* in different world regions is important not only from an ecological point of view but also in terms of health risk assessment. Over the years, numerous experimental and in-field studies on *C. raciborskii* have been conducted by Australian, Asian, North and South American and European research teams (for reviews see Bonilla et al. 2012; Rzymski and Poniedziałek 2014; Antunes et al. 2015; Burford et al. 2016). Investigations in Poland have contributed to a general understanding of *C. raciborskii* in the temperate zone although a number of unknowns remain that require further attention.

**Distribution in Poland**

*C. raciborskii* was described by Woloszyńska from the island of Jawa (Wołoszyńska 1912) and for many years it was considered as a tropical and subtropical species. In Europe it was first reported in Lake Kastoria in Greece (Skuja 1937). It is postulated that the expansion of *C. raciborskii* toward higher latitudes occurred at the end of the 20th century. The species is widely distributed throughout Europe (Antunes et al. 2015) and recently, it was also reported in the very north-eastern part of Europe in the highly eutrophic Lake Nero in The Yaroslavl Region, Russia (Babanazarova et al. 2015). Therefore, it appears that its northward spread continues. Different theories explaining this phenomenon have been put forward including global warming, phenotypic plasticity or the occurrence of physiologically different ecotypes (Bonilla et al. 2016; Burford et al. 2016).

In Poland *C. raciborskii* was documented for the first time in the 1970s in the artificially heated Lake Pątnowskie near Konin in the eastern part of the Wielkopolska region (Burchardt 1977). This lake constitutes part of the cooling system for an electric power plant and receives warm waters discharged throughout the year resulting in steadily increased lake water temperature. Recently, *C. raciborskii* was reported in the second water body of this cooling system, Lake Licheńskie (Burchardt et al. 2014) which is characterized by the highest water temperature (reaching 30°C) and never forming ice cover (Napiórkowska-Krzębińeczke 2009). The occurrence of this cyanobacterium has also been reported in several thermally normal lakes of Western Wielkopolska (Stefaniak and Kokociński 2005), including water bodies located in the Wielkopolski National Park (Pełechata and Ławniczak 2013). Its occurrence was also found in the low-land river Samica Stęszewska (Pełechata and Ławniczak 2013). This right tributary of the Warta River flows through the lakes in which *C. raciborskii* was documented (Kokociński et al. 2013), thus representing a potential route of dispersion.

Large-scale spatial studies have revealed the common occurrence of *C. raciborskii* in the western part of Poland and increasing contribution in total phytoplankton biomass in the investigated lakes (Kokociński and Soininen 2012). Observations of this species have also been reported from the Lubuskie Lakeland in mid-western Poland (Pełechata et al. 2006), Kujawsko-Pomorskie Province in north-central Poland (Kokociński et al. 2013)) and from Lake Rekąty and Lake Piłwąg situated in the region of the Great Mazurian Lakes in northeastern Poland (Jakubowska et al. 2013). However, recent papers concerning cyanobacteria studies from other regions of Poland do not report of the presence of this species (Kobos et al. 2013; Bukowska et al. 2014). In addition, the latest spatial studies in Poland and Lithuania revealed that the occurrence of *C. raciborskii* is limited to the western part of Poland and a single presence in Lithuania (Kokociński et al. 2016). It generally occurs in turbid, shallow, eutrophic lakes with increased concentration of phosphorus and nitrogen (Kokociński and...
Soininen 2012) while its contribution to the total phytoplankton biomass is usually not high, even during cyanobacterial blooms, and maximally does not exceed 30% (usually being considerably lower) (Kokociński et al. 2013). Moreover, its numerous occurrence is limited to the warmest summer months, although single filaments have also been observed during colder temperatures in late September and October (Brygider and Kokociński 2016). The long term phytoplankton analysis in Poland revealed the ability of *C. raciborskii* to outcompete a native bloom forming cyanobacterial species *Planktothrix agardhii* (Kokociński et al. 2010). It was also experimentally evidenced that the Polish strain of *C. raciborskii* can outcompete *Microcystis aeruginosa* even at relatively low initial biomass (Rzymski et al. 2014).

**Potential toxicity**

*C. raciborskii* is a potent toxin producer. This feature is not only geographically-diversified (Rzymski and Poniedziałek 2014) but particular strains from the same region can also vary in this respect (Chonudomkul et al. 2004). Furthermore, particular populations may be constituted of non-toxic and toxic strains co-occurring within the same habitat while toxic-positive strains may also vary in levels of biosynthesized toxin (Willis et al. 2016).

The first toxic compound ever identified (in 1992) for *C. raciborskii* was CYN, a polyketide-derived alkaloid with a central functional guanidino moiety combined with hydroxymethyluracil attached to its tricyclic carbon skeleton (Poniedziałek et al. 2012). Later, other species were also identified as potent CYN-producers. The toxin is actively released by intact cells to the extracellular environment (Preußel et al. 2009), remaining relatively stable over a wide range of pH and temperature (Chiswell et al. 1999); it can accumulate in the water column and has been found in sediments from approximately 4700 years ago (Waters 2016). CYN has been reported to be produced by *C. raciborskii* strains from Australia and New Zealand (Hawkins et al. 1997; Wood and Stirling 2003), and Asia (Lei et al. 2014). To date no European strain of *C. raciborskii*, including Polish strains, have been identified as either producers of CYN or possessors of genes involved in its biosynthesis (Antal et al. 2011; Kokociński et al. 2013; Rzymski et al. 2017). Other cyanobacterial producers of CYN were, however identified in Europe, e.g. *Aphanizomenon gracile* in Poland (Kokociński et al. 2013) or *Oscillatoria* sp. in France (Mazmouz et al. 2010). Although one recent study has reported the alleged production of CYN by *C. raciborskii* blooming in Lake Aleksandrovac in Serbia, the whole conclusion was not based on any molecular and/or analytical investigation of the isolated strain (Đorđević et al. 2015). Moreover, Svirčev et al. 2016 found that the strain from this reservoir exhibits toxicity in *Artemia salina* bioassays but does not produce CYN, STX or microcystin (MC).

The second important group of toxins which are known to be produced by *C. raciborskii* are STXs. However, their production has only been confirmed for strains from South America (Rzymski and Poniedziałek 2014). The presence of STX was reported in a bloom dominated by *C. raciborskii* in Greece but convincing evidence is insufficient due to a lack of molecular and analytical studies conducted on the isolated strain (Gkelis and Zaatouss 2014). Nevertheless, other nostocalean cyanobacteria are known to be responsible for STX production (Ballot et al. 2010; Cirés et al. 2014). STXs are neurotoxic alkaloids that block voltage-gated sodium channels in neuronal cells, also implicated in paralytic shellfish poisoning (O’Neill et al. 2016). To date, over 55 analogues that vary in toxicity have been identified including non-sulfated, mono-sulfated, di-sulfated, decarbamoylated and hydrophobic variants (Wiese et al. 2010).

Interestingly, one strain from Tunisia (Bir M’cherga reservoir) was reported to possess two segments from the *mcy* gene cluster (*mcyA* and *mcyE* genes) involved in the production of MC, a cyclic peptide hepatotoxin although the concentrations of toxin were below the detection limit (Fathalli et al. 2011). Most recently, a preliminary report on the production of MC by a Greek strain of *C. raciborskii* has been demonstrated (Panou et al. 2016).

It was also lately found that North American strains of *C. raciborskii* are able to produce lipophilic congeners of PMA which exert toxic and specifically, teratogenic activity in the zebrafish embryo (Jaja-Chimedza et al. 2012; Jaja-Chimedza et al. 2015). To date, no strains from other geographical regions have been screened for the production of PMAs. Such studies are imperative given the fact that a number of European strains of *C. raciborskii* were found to display in vitro and in vivo toxicity but no known cyanotoxins were identified. For example, extracts from German isolates were toxic to primary rat hepatocytes, human hepatoblastoma and human colon adenocarcinoma cells (Fastner et al. 2003). Exposure of rodents to a French strain of *C. raciborskii* caused liver damage (Bernard et al. 2003). Hepatotoxicity as well as neurotoxic effects (lethargy, piloerection, and difficulty in breathing) were observed in mice exposed to crude extracts of a Portuguese strain (Saker et al. 2003). In turn, extracts of Lake Balaton strains were reported to be toxic in four bioassays: *Thaunocephalus platyurus* acute lethality test; *Daphnia magna* acute immobilization assay; *D. magna* feeding inhibition assay and *Danio rerio* embryo developmental toxicity assay.
Moreover, retinoid-like activity provoking teratogenic effects in frog embryos was recently found for one strain from Lake Balaton (Smutná et al. 2016). Other Hungarian strains were, in turn, shown to evoke neurotoxicity involving inhibition of the acetylcholine responses in Helix pomata (Vehovszky et al. 2015). Toxic effects were also found for Polish strains of C. raciborskii (see “Toxicity of Polish strains” section). The state-of-the-art of the toxic potential of European strains of C. raciborskii is summarized in Figure 2.

Toxicity of Polish strains

Toxicological studies employing an in vitro experimental model revealed that certain strains of C. raciborskii from Western Poland can produce toxic compound(s). Specifically, cell-free extracts obtained from these strains were found to decrease proliferation and induce apoptosis in human T-lymphocytes (Poniedziałek et al. 2015) and cause oxidative stress in human neutrophils, eventually leading to lipid peroxidation and decreased cell survival (Rzymski et al. 2017). Importantly, adverse responses were observed in time as short as 1 h indicating that the potential risk arising from the produced toxin(s) is rather high (Poniedziałek et al. 2015; Rzymski et al. 2017).

To date, Polish strains have not been found to produce any known toxic metabolites although screening of a relatively wide number of metabolites was conducted (on an analytical and molecular level) and included CYN, STXs, guanidineacetate, anatoxin-a (ANA-A), α-γ-diaminobutyric acid (DAB), β-N-methylamino-L-alanine (BMAA) and MC (Mankiewicz-Boczek et al. 2012; Kokociński et al. 2013; Rzymski et al. 2017).

In another study, extracts obtained from the Polish strain of C. raciborskii induced the production of alkaline phosphatase, a response similar to that observed for CYN (isolated from the Australian strain of C. raciborskii) and it was suggested that it may be involved in allelopathic interactions (Rzymski et al. 2014). However, this strain was not found to contain genes from the cyr cluster, including cyrA which encodes the product associated with the initial reaction, transformation of guanidineacetate substrate, in CYN biosynthesis (Rzymski et al. 2017). Further studies are necessary to identify compounds produced by Polish strains of C. raciborskii which are involved in reported toxicity and allelopathic effects.

Conclusions

C. raciborskii is frequently found in the lakes of western Poland although it does not usually reach high frequency in the phytoplankton community and does not form blooms under current environmental conditions. Similarly to findings from other areas of Europe, Polish strains of this species are able to produce toxic compound(s) although no synthesis of CYN, MC, ANA-A, STX, BMAA and DAB was found. The toxic compounds have yet to be identified.

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References


Bananazaraova O.V., Sidelev S.I., Fastner J., 2015, Northern expansion of *Cylindrospermopsis raciborski* (Nostocales, Cyanobacteria) observed in shallow highly eutrophic lake Nero (Russia), Int. J. Algae 17(2): 131–141.


Brygider A., Kokociński M., 2016, *Cylindrospermopsis raciborskii* – a growing ability of tropical cyanobacterium to adapt to conditions in temperate lakes of Western Poland (northern Greater Poland, lake Pniewskie and Kierskie Małe) [Abstract], Abstract book of the 11th International Conference of Young Naturalists “From Biotechnology to Environmental Protection” – Interdisciplinary Meeting of Young Naturalists, Zielona Góra, Poland, 23–26 November.


Jaja-Chimda A., Gantar M., Gibbs P.D., Schmale M.C., Berry J.P., 2012, Polyetheroxy-1-alkenes from Aphanozomenon ovalisporum inhibit vertebrate development in


Pöndzialek B., Ryzmiski P., Kokociński M., Karczewski J., 2015, Toxic potencies of metabolite(s) of non-cylindrospermopsin producing Cylindrospermopsis raciborskii isolated from temperate zone in human white cells, Chemosphere 120: 608–614.


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**mopsis raciborskii** effect on the growth and metabolism of *Microcystis aeruginosa*, Harmful Algae 35: 1–8.


