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Short communication

Intraspecific variation in growth, morphology and toxin quotas for the cyanobacterium, *Cylindrospermopsis raciborskii*

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ABSTRACT

Cylindrospermopsis raciborskii is a bloom forming cyanobacterium with complex population dynamics and toxicity. In January of 2013 a single sample was collected from surface waters in Lake Wivenhoe, Australia, and twenty-four individual trichomes were isolated. Each isolate exhibited differences in growth rate, toxin cell quota and morphology, in the absence of phylogenetic heterogeneity. This study demonstrates substantial intraspecific isolate variation within a small sample and this has implications for understanding the population dynamics of this species.

Cylindrospermopsis raciborskii is an invasive freshwater cyanobacterium of global importance (Burford et al., 2016). Despite very low phylogenetic heterogeneity amongst isolates, strains can exhibit differences in morphology, with either straight or coiled trichomes, as well as differences in growth rates, nutrient metabolism, and toxin production (Chonudomkul et al., 2004; Piccini et al., 2011; Saker and Griffiths, 2000). *C. raciborskii* is attributed with the production of the toxins cylindrospermopsin (CYN) and saxitoxin (SXT). Toxin production is strain dependent and reflects biogeographical distributions, with Australian, New Zealand and Chinese strains producing CYN, South American strains producing SXT, and European and North American strains typically producing neither (Antunes et al., 2015).

The geographical distribution or dominance of *C. raciborskii* appears to be expanding from tropical to subtropical and temperate regions (Sinha et al., 2012; Wood et al., 2014). Due to differences in toxin production between strains, the risk from blooms is difficult to predict (Orr et al., 2010; McGregor and Fabbro, 2000; Hamilton et al., 2014).

C. raciborskii strains isolated from several geographical locations within a region or country show considerable variation in growth response to environmental conditions such as temperature, light, and nutrients (Amaral et al., 2014; Pierangelini et al., 2014a,b; Saker and Griffiths, 2000; Willis et al., 2015). Such variations reflect a highly adaptive strategy and are speculated to be the main driver for the rapid geographic expansion of *C. raciborskii* (Sinha et al., 2012). However, the scale at which this diversity exists is unclear, including within a single population in a waterbody (Saker et al., 1999; Burford et al., 2014). This study examined diversity by assessing the phylogeny, growth rate, toxin quota/ratios and morphological variations between isolates from a single population in a subtropical reservoir.

Two hundred millilitres of surface water was collected from Lake Wivenhoe, (27.3939° S, 152.6078° E, Queensland, Australia, collected on 16 January 2013), immediately returned to the laboratory, and enriched with 50 mL of Jaworski's medium (Thompson et al., 1988). Twenty four *C. raciborskii* trichomes were isolated using a combination of micro-manipulation and serial dilution. The isolates were grown in 250 mL tissue culture flasks under 12 h:12 h light:dark cycle, with 15 μmol photons m^{-2} s^{-1}, at 28 °C, once cultures reached 6 × 10^5 cells mL^{-1}, they were split into triplicate cultures. Each isolate was characterised with light microscopy

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(Leica DM4000) for trichome morphology (straight or coiled), cells trichome^{-1}, and measurements of cell width and length. Cell biovolume was calculated from the formula for a cylinder cell shape using average cell measurements, according to Hillebrand et al. (1999). Culture density of triplicate cultures was measured on alternate days by optical density at 750 nm using a spectrophotometer (Novaspec II, Pharmacia Biotech), over a 30 day growth cycle. Maximum growth rate was determined during exponential growth phase (Andersen, 2005).

A 5 mL culture sub-sample and a 5 mL filtered sub-sample were collected from the triplicate cultures in late exponential growth phase (day 15), both samples were lyophilised and CYN and deoxy-CYN (dCYN) extracted and analyzed by HPLC-MS as previously described (Willis et al., 2015). The CYN cell quota (Q_{CYNs}) was calculated from the total intracellular CYNs (culture sample − filtered sample; CYN + dCYN) divided by the cell number (Hotzel and Croome, 1999). The ratio of CYN:dCYN was determined for the intracellular toxin. One-way ANOVAs performed in Excel (Microsoft) were used to determine statistical distances between strains for each measurement. Clustering of the isolates according to growth rate, toxin quota/ratios and morphological characteristics was made by estimating the Euclidean distance between isolates on the basis of standardised measurements of trichome length, cell biovolume, growth rate, Q_{CYN} and the CYN:dCYN ratio.

Genomic DNA was isolated from cultures by phenol/chloroform extraction (Ausubel et al., 2003) and quality and quantity was assessed by spectrophotometry and by agarose gel electrophoresis. Phylogenetic discrimination of the 24 *C. raciborskii* isolates was made using HIP (HIP_1) markers as previously described (Neilan, 1995; Saker and Neilan, 2001).

Of the 24 *C. raciborskii* isolates, no two exhibited identical growth rate, toxin quota/ratios and morphological characteristics (Table 1). Statistical clustering of the isolates revealed less variation of growth rate, toxin quota/ratios amongst coiled than straight isolates (Fig. 1). Amongst coiled isolates two groups emerged with those possessing longer trichomes and higher Q_{CYNs} (C01, C02, C05) differentiated from those with higher growth rates and greater cell biovolumes (C03, C04, C06, C07). Amongst the straight isolates, two isolates (S10 and S17) were distinct from other isolates. S10 possessed very high biovolumes and S17 possessing very long filaments. The remaining isolates could be differentiated on the basis of high Q_{CYNs} (S01-04, S11-S16) and high CYN:dCYN ratios (S05-S09). These two measures were inversely correlated ($r = -0.508$, $p = 0.011$) across all isolates.

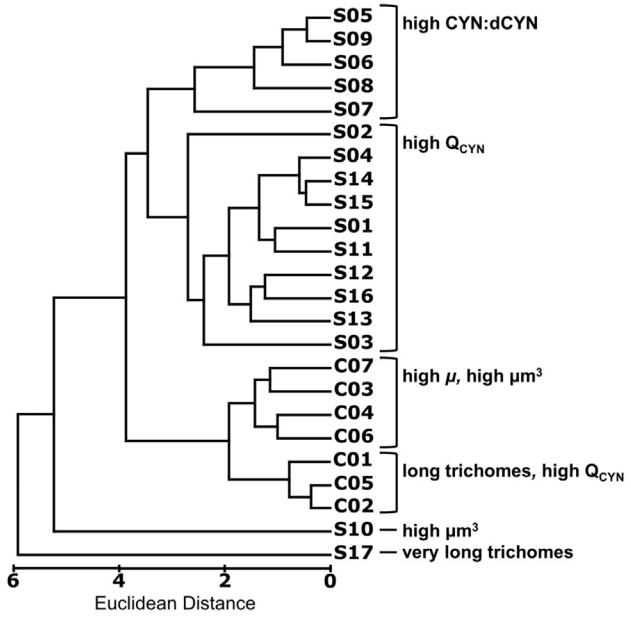

Fig. 1. Euclidean distance phylogeny of the 24 isolates, based on growth rate, toxin quota/ratios and morphological characteristics.

Significant variance ($p < 0.001$) for all characteristics was measured between the isolates (Table 1): with cell volume ranging from 30 to 263 μm^3; maximum growth rate ranging from 0.10 to 0.21 d^{-1}; Q_{CYNs} ranging from 95 to 232 fg cell^{-1}, cells trichome^{-1} ranging from 5 cells to 27 cells trichome^{-1}; and CYN:dCYN ratio ranging from 5% to 50% dCYN.

Table 1
Growth rate, toxin quota/ratios and morphological characterisation of the 24 *Cylindrospermopsis raciborskii* isolates.

Isolate	Trichome morphology	Cells trichome^{-1}	Cell biovolume (μm^3)	Growth rate (d^{-1})	Q_{CYNs} (fg CYN + dCYN cell^{-1})	CYN:d-CYN
S01	Straight	10 ± 2.54	50.87 ± 17.74	0.12 ± 0.01	152.55 ± 12.33	0.66 ± 0.02
S02	Straight	8 ± 2.6	29.95 ± 12.06	0.10 ± 0.02	225.41 ± 7.59	0.82 ± 0.0
S03	Straight	8 ± 2.83	57.51 ± 18.00	0.17 ± 0.04	278.88 ± 16.68	0.67 ± 0.01
S04	Straight	9 ± 2.9	46.03 ± 10.93	0.15 ± 0.06	184.96 ± 7.84	0.84 ± 0.02
S05	Straight	6 ± 2.03	57.09 ± 26.16	0.15 ± 0.00	94.60 ± 5.16	4.92 ± 0.59
S06	Straight	6 ± 2.35	75.81 ± 51.11	0.15 ± 0.01	126.34 ± 10.62	5.43 ± 0.28
S07	Straight	8 ± 2.16	102.81 ± 50.15	0.11 ± 0.01	214.09 ± 12.60	4.6 ± 0.06
S08	Straight	5 ± 1.53	71.32 ± 27.53	0.11 ± 0.01	119.79 ± 3.77	5.45 ± 0.42
S09	Straight	6 ± 1.91	67.05 ± 27.2	0.14 ± 0.02	90.89 ± 2.76	4.7 ± 0.18
S10	Straight	7 ± 2.41	262.93 ± 107.97	0.13 ± 0.01	201.37 ± 4.18	0.7 ± 0.02
S11	Straight	10 ± 2.82	82.66 ± 32.05	0.14 ± 0.01	140.45 ± 4.58	1.05 ± 0.07
S12	Straight	10 ± 2.47	63.62 ± 44.43	0.18 ± 0.01	205.44 ± 10.04	0.57 ± 0.02
S13	Straight	10 ± 2.61	125.35 ± 53.19	0.17 ± 0.02	187.22 ± 15.13	0.74 ± 0.07
S14	Straight	10 ± 3.46	61.68 ± 24.85	0.14 ± 0.01	204.65 ± 11.61	0.59 ± 0.02
S15	Straight	10 ± 2.83	62.84 ± 47.89	0.15 ± 0.01	193.16 ± 6.99	0.81 ± 0.02
S16	Straight	11 ± 3.77	84.29 ± 25.3	0.17 ± 0.01	232.31 ± 13.09	2.15 ± 0.08
S17	Straight	27 ± 12.04	82.89 ± 35.94	0.15 ± 0.01	165.56 ± 13.16	4.37 ± 0.41
C01	Coil	11 ± 3.67	32.51 ± 9.3	0.15 ± 0.01	182.65 ± 35.75	1.00 ± 0.06
C02	Coil	11 ± 2.69	39.15 ± 12.3	0.17 ± 0.01	178.01 ± 14.39	1.00 ± 0.07
C03	Coil	6 ± 2.15	55.27 ± 31.94	0.21 ± 0.01	121.13 ± 5.50	1.72 ± 0.01
C04	Coil	7 ± 2.82	63.37 ± 17.9	0.18 ± 0.01	115.30 ± 17.73	1.73 ± 0.02
C05	Coil	10 ± 3.05	35.85 ± 42.69	0.17 ± 0.00	166.28 ± 53.96	1.08 ± 0.03
C06	Coil	10 ± 2.30	62.49 ± 30.11	0.18 ± 0.01	143.73 ± 8.92	1.16 ± 0.01
C07	Coil	7 ± 3.19	51.36 ± 15.87	0.21 ± 0.00	163.32 ± 6.10	0.58 ± 0.01