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Polyphasic identification of cyanobacterial isolates from Australia

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ABSTRACT

Reliable identification of cyanobacterial isolates has significant socio-economic implications as many bloom-forming species affect the aesthetics and safety of drinking water, through the production of taste and odour compounds or toxic metabolites. The limitations of morphological identification have promoted the application of molecular tools, and encouraged the adoption of combined (polyphasic) approaches that include both microscopy- and DNA-based analyses. In this context, the rapid expansion of available sequence data is expected to allow increasingly reliable identification of cyanobacteria, and ultimately resolve current discrepancies between the two approaches.

In the present study morphological and molecular characterisations of cyanobacterial isolates ($n = 39$), collected from various freshwater sites in Australia, were compared. Sequences were obtained for the small ribosomal subunit RNA gene (16S rDNA) ($n = 36$), the DNA-dependent RNA polymerase gene (*rpo*C1) ($n = 22$), and the phycocyanin operon, with its intergenic spacer region (*cpc*BA-IGS) ($n = 19$). Phylogenetic analyses identified three cyanobacterial orders: the Chroococcales ($n = 8$), Oscillatoriales ($n = 6$), and Nostocales ($n = 25$). Interestingly, multiple novel genotypes were identified, with 22% of the strains (17/77) having <95% similarity to available sequences in GenBank.

Morphological and molecular data were in agreement at the species level for only 26% of the isolates obtained (10/39), while agreement at the genus level was obtained for 31% (12/39). Confident identification of the remaining 44% of the strains (17/39) beyond the order level was not possible. The present study demonstrates that, despite the taxonomic revisions, and advances in molecular-, and bioinformatics-tools, the lack of reliable morphological features, culture-induced pleomorphism, and proportion of misidentified or poorly described sequences in GenBank, still represent significant factors, impeding the confident identification of cyanobacteria species.

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1. Introduction

Cyanobacteria are a group of ubiquitous photosynthetic prokaryotes, found in all types of aquatic environments (Whitton and Potts, 2000). Interest in this phylum has increased due to (i) their ancient evolutionary origins (Tomitani et al., 2006), (ii) their ecological role as oxygen producers, and atmospheric nitrogen- and carbon-fixers (Reynolds, 2006), (iii) the socio-economic impact on various industries (e.g. water, tourism and food) of bloom-forming producers of toxins and/or odorous metabolites (Granéli and Turner, 2006), and (iv) their application as a source of biofuels and pharmaceuticals (Borowitzka, 1995; Li et al., 2008).

Cyanobacteria identification, enumeration and classification have traditionally been based on light-microscopy observations, using morphological characteristics such as cell size, cell fission type, trichome width, shape of the terminal cells, shape, size and position of specialised cells such as akinetes and heterocytes, presence of aerotopes etc. (Castenholz, 2001). However, this approach requires considerable operator skill and time; with distinctive phenotypic characteristics varying significantly within species, or even being lost, due to environmental or culture conditions, growth phase, use of fixatives etc. (Lyra et al., 2001; Whitton and Potts, 2000). Furthermore, manifestation of ecotypes, or microbial pleomorphism during long-term cultivation, has resulted in a large number of strains being misidentified, with disagreeing nomenclature and morphological descriptions (Komárek, 2006, 2010).

The well-known limitations to morphology-based identification promoted the development of DNA-based approaches, as a means of reliably identifying cyanobacterial isolates (Valério et al., 2009; Willame et al., 2006). The small ribosomal subunit RNA gene (16S rDNA), together with its internal transcribed spacer (ITS) region, have been widely used for taxonomic purposes, to profile complex prokaryotic communities, and infer phylogenetic relationships (Castenholz, 2001; Coenye and Vandamme, 2003; Komárek, 2006). Other commonly used loci include the more discriminatory protein-coding gamma subunit of the DNA-dependent RNA polymerase (*rpo*C1) (Fergusson and Saint, 2000), and the phycocyanin operon, consisting of the two *cpc*B-*cpc*A genes with their variable intergenic region (PC-IGS) (Neilan et al., 1995).

Previous studies have shown that identification of unknown isolates can be hampered by incomplete (or unreliable) morphological descriptions being provided for the sequenced strains, incorrect identification of strains in culture collections, and/or simply, by the lack of proper reference strains *tout court* (Komárek, 2006, 2010; Rajaniemi et al., 2005). Much has been done to overcome these problems, such as the proposal of the International Code of Nomenclature for algae, fungi, and plants (Castenholz and Norris, 2005; McNeill et al., 2012; Oren, 2004, 2011; Oren and Tindall, 2005), and the International Code of Nomenclature of Bacteria which groups cyanobacteria into subsections (Castenholz, 2001), and the resulting revisions to cyanobacteria nomenclature and classification (Anagnostidis, 2001; Otsuka et al., 2001; Rajaniemi et al., 2005; Suda et al., 2002). However, this has also resulted in new taxa being described using a combination of both codes, causing further confusion (Komárek, 2010).

With time, the rapid expansion of available sequence data (Fig. 1) is expected to allow increasingly accurate molecular identification of cyanobacteria, and help in resolving the discrepancies between the microscopy and molecular approaches. In light of the current maturity of sequence databases, taxonomy and molecular tools (Komárek et al.,

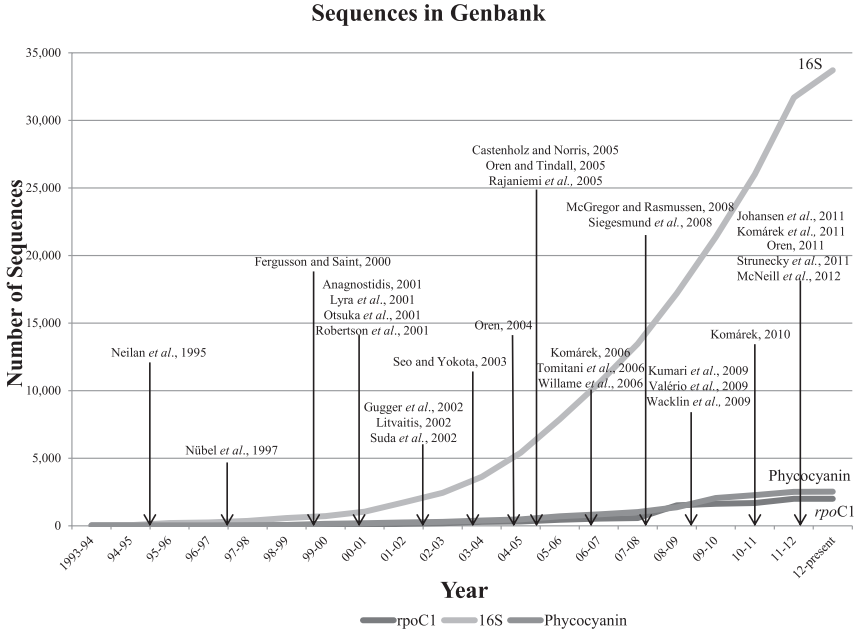

Fig. 1 – Number of sequences within GenBank for the 16S, *rpo*C1 and phycocyanin loci over time. Data obtained from NCBI Nucleotide Database (http://www.ncbi.nlm.nih.gov/nuccore). References indicate papers characterising or reporting changes to cyanobacteria taxonomy and nomenclature.