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Homogeneous detection of cyanobacterial DNA via polymerase chain reaction

C.E. Lane[1], M.T. Gutierrez-Wing[2], K.A. Rusch[2] and M.G. Benton[1]

1 Cain Department of Chemical Engineering, Louisiana State University, Baton Rouge, LA, USA
2 Department of Civil & Environmental Engineering, Louisiana State University, Baton Rouge, LA, USA

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Correspondence
Michael G. Benton, Cain Department of Chemical Engineering, 110 Chemical Engineering, South Stadium Drive, Baton Rouge, LA 70803, USA.
E-mail: benton@lsu.edu

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Abstract

Aims: To design a primer set enabling the identification through PCR of high-quality DNA for routine and high-throughput genomic screening of a diverse range of cyanobacteria.
Methods and Results: A codon-equivalent multiple alignment of the phycocyanin alpha-subunit coding sequence (*cpcA*) of 22 cyanobacteria was generated and analysed to produce a single degeneracy primer set with virtually uniform product size. Also, an 18S ribosomal RNA detection set is proposed for rejecting false positives. The primer sets were tested against five diverse cyanobacteria, *Chlorella vulgaris*, *Saccharomyces cerevisiae*, and *Escherichia coli*. All five cyanobacteria showed positive amplification of *cpcA* product with homogeneous fragment length, and no products were observed for any other organism. Additionally, the only product formation observed for the 18S rRNA set was in *C. vulgaris* and *S. cerevisiae*.
Conclusions: The newly proposed primer set served as effective check primers for cyanobacteria. Cyanobacteria gDNA had a positive, homogenous result, while other bacteria, eukaryotes and alga tested were negative.
Significance and Impact of the Study: These novel, broad-spectrum primers will greatly increase the utility of PCR on newly discovered cyanobacterial species.

Introduction

Cyanobacteria produce a variety of bioactive metabolites and commodity 'bio-products' such as isoprene, biofuels and biopolymers at nearly carbon-neutral conditions (Ducat *et al.* 2011). These metabolites exhibit a variety of properties from anti-(viral, fungal, bacterial) (Abed *et al.* 2009) to immunosuppressive (Abed *et al.* 2009) and even anti-cancer (Simmons *et al.* 2005).

A major deterrent to industrial-scale bio-production is the cost of the carbon feedstock, limiting the economic viability of bio-products *vs* products produced by more traditional methods (Lee *et al.* 1999; Ducat *et al.* 2011). Because cyanobacteria have the ability to fix carbon through photosynthesis, such financial concerns could be attenuated. With millions of cyanobacteria strains worldwide, there is a distinct possibility that currently uncharacterized species will enhance production of specific bio-products. Therefore, the isolation and characterization of new cyanobacterial species could lead to the increased production of environmentally friendly, sustainable commodities.

Ideally, one could isolate a new cyanobacterial species and rapidly characterize its commodity production potential via a routine, quick assay such as PCR. Unfortunately, DNA-based techniques such as PCR are complicated when dealing with cyanobacteria, because cyanobacteria are morphologically diverse, and typical DNA isolation techniques are not always effective. Therefore, before PCR can be successfully used for cyanobacteria screening, verification that intact, genomic cyanobacterial DNA has been successfully isolated is a must.

Traditionally in PCR, check primers are used to verify successful DNA isolation. If a PCR primer binds successfully and amplification occurs, one can be confident that a valid template strand (often genomic DNA) is present. Often, there is a single housekeeping gene used to generate check primers, thereby serving as a positive control

for multiple strains within a given organism class. For example, 18S rRNA genes are commonly used in yeast (Lantz *et al.* 1999) and the glutamate decarboxylase and β-D-glucuronidase genes in *Escherichia coli* (McDaniels *et al.* 1996). Generally, housekeeping genes targeted in PCR include those encoding ribosomal RNA, actin, tubulin, ubiquitin and elongation factors (Jain *et al.* 2006; Filby and Tyler 2007; Garg *et al.* 2010).

Check primers for the cyanobacteria phylum have been designed in one of two ways. The first method is to target a gene or operon that is uniquely specific to the phylum of interest. For example, in cyanobacteria the abundance of the phycobiliprotein phycocyanin (C-PC), one of two blue photosystem accessory pigments, aids chlorophyll *a* energy harvesting in photosynthesis (MacColl 1998). In the model cyanobacterium *Synechocystis* sp. PCC 6803 it is encoded by a five gene operon (Ughy and Ajlani 2004). Two genes of importance (*cpcA* and *cpcB*) code for the phycocyanobilin-binding subunits (αPC and βPC respectively), while the other three genes code for rod linker polypeptides (Ughy and Ajlani 2004). The amino acid that binds the chromophore via thioester linkage is very well conserved (Cys_{84} in αPC) (MacColl 1998). This operon is found primarily in cyanobacteria, but also in some cryptophyta and rhodophyta plastids (MacColl *et al.* 1999; Eriksen 2008). Neilan *et al.* have shown the α/β intergenic spacer (IGS) is a novel region to investigate for phylogenetic classification of cyanobacteria because of its variability (Neilan *et al.* 1995). However, they report heterogeneity in amplification products across cyanobacterial strains (500–740 bp product) and no amplification product in *Nostoc punctiforme* PCC 73102 and *Nostoc commune* NIES 24 despite multiple reaction conditions (Neilan *et al.* 1995).

The second method targets a universal gene and achieves specificity through exploitation of cyanobacteria-specific consensus regions. An excellent example is the 16S ribosomal RNA primer sets developed by Nübel *et al.* (1997). The original intent of these sets, similar to those of Neilan and coworkers, was to show diversity within cyanobacterial populations using denaturing gradient gel electrophoresis (DGGE) of the PCR product fragments and for this purpose included up to 40-mer regions of GC-rich 5′ tails in up to 62-bp primer length (Nübel *et al.* 1997). While the CYA359F/CYA781R(a and b mixture) set have been proven discriminant for cyanobacteria and plastids (Nübel *et al.* 1997), direct amplification using these primers have been shown to produce weak signal when investigated by Boutte *et al.* (2006). As a consequence of this weak signal, it has become typical when using these primers to perform semi-nested PCR with additional oligos in order to increase the product amplification (Boutte *et al.* 2006; Lymperopoulou *et al.* 2011). It should be noted that a variation of the primer set developed by Nübel *et al.* has been proposed by McGregor and Rasmussen; however, to our knowledge, there has been no evidence of it recently being used as a cyanobacteria-specific PCR control (McGregor and Rasmussen 2008). Also, an oxyphotobacteria-specific 16S ribosomal RNA primer set has been proposed by Rudi *et al.* (1997). While they observed positive amplification in cyanobacteria, including *Nostoc*, they too observed best results when performing semi-nested PCR (Rudi *et al.* 1997). The two sets developed by Neilan *et al.* and Nübel *et al.* are among the most commonly used controls in cyanobacteria-related PCR (Vaitomaa *et al.* 2003; Saker *et al.* 2007).

For high-throughput genetic screening and/or development of novel DNA isolation techniques for problematic strains, the primer sets described above have limitations when considered collectively. For example, some sets have shown weak signal, which can lead to false negatives or inconclusive results. Although this weakness is sometimes overcome with semi-nested PCR, this also is not ideal because semi-nested PCR requires two separate (nontandem) reactions, a more expensive and time-consuming process. Also, the sets have failed to detect certain species, limiting their utility as check primers for general cyanobacterial applications, especially with nonidentified strains.

To address some of the limitations associated with the check primers above, we propose a new primer set capable of detecting high-quality cyanobacterial DNA in a single-step reaction, even when little-to-no sequence information is available. The proposed set amplifies the majority of the *cpcA* gene. The design effectively reduces the chances of false-negative detection with respect to PCR amplification and generates a single approximate product length. Also proposed is a general detection set to amplify the 18S small subunit (SSU) ribosomal RNA gene in order to test for the presence of eukaryotic DNA in efforts to prevent false positives from eukaryotic alga. According to the NCBI Taxonomy database (accessed 2012), currently 86% of the entries in cyanobacteria can be accounted for by only three classes: Nostocales, Chroococcales and Oscillatoriales (2002). Therefore, representative species for each of the three major classes were tested using the proposed *cpcA* and 18S rRNA gene detection sets.

Materials and methods

Synechocystis sp. PCC 6803, *Plectonema* sp. UTEX 1541, *Nostoc muscorum* UTEX 1037, *N. punctiforme* UTEX B1629, *Spirulina platensis* UTEX LB2340 were cultivated in a Forma Scientific Plant Tissue Culture Incubator Model 3750 at 29°C under fluorescent lighting in BG-11 medium (Rippka *et al.* 1979) supplemented with

100 mmol l^{-1} TES buffer (pH 8·2). The 25-ml cultures, contained in 50-ml polypropylene tubes with loosely fitted lids, were agitated once daily.

The *Chlorella vulgaris* used was a unialgal strain isolated from college lake near the LSU campus (Rusch). It was grown at room temperature 0·5 m from overhead fluorescent lighting in f-medium (Guillard 1975) in a 500-ml borosilicate media bottle with a loosely fitted lid and agitated once daily.

Saccharomyces cerevisiae BY4741 (originally derived from S288C) and *E. coli* DH5-α were grown in the New Brunswick Innova 4340 shaking incubator at 150 rev min^{-1} and under no illumination. These 6 ml cultures in 15-ml polypropylene tubes were cultured in YPD medium (Sambrook 2001) and Luria-Bertani (LB) medium (Sambrook 2001), respectively.

Synechocystis sp., *Plectonema* sp., *S. platensis* and *N. muscorum* cell concentrations were estimated by microscopy (Zeiss Axio Observer; Carl Zeiss MicroImaging GmbH, Göttingen, Germany) using a Hausser Scientific hemocytometer with improved Neubauer ruling (0·1-mm chamber depth). These concentrations were correlated to spectrophotometric absorbance at 730 nm (Williams 1988) measured using a Beckman Coulter DU730 UV/Vis spectrophotometer. Growth phase was approximated using spectrophotometric measurements and these correlations. To aid the counting of *N. muscorum*, a 25 ga. syringe was used to better separate the filaments from the large aggregate mats. Because of the high difficulty of precisely quantifying the matting cyanobacteria, *N. punctiforme* was assumed analogous to *N. muscorum*, as these two strains had highly similar morphology, and only order of magnitude estimates of cell concentration were necessary to perform DNA isolations.

The total genomic DNA (gDNA) of most cyanobacteria were extracted in the mid-to-late exponential growth phase using a protocol described by Neilan (2002). However, an optimized protocol described by Morin *et al.* (2010) provided a more robust gDNA isolation technique that was required for *S. platensis*.

Total gDNA was isolated from *S. cerevisiae*, *E. coli* and *C. vulgaris* using the Bust n' Grab method (Harju *et al.* 2004). The *C. vulgaris* gDNA extraction was performed during the late stationary phase, and an additional chloroform/isoamyl alcohol (24 : 1) purification step was performed on the DNA isolate sample.

All gDNA template samples were analysed via spectrophotometry using the Beckman Coulter NanoVette (0·2-mm path length) and diluted to a final concentration of 50 ng μl^{-1} in TE buffer (pH 8·0) (Sambrook 2001). Because this paper proposes a primer set to be used in high-throughput methods, RNAse steps were omitted from all protocols to ensure primer effectiveness in the presence of 'dirty' samples typical of such methods.

The primary structure for both αPC and *cpcA* from 22 various cyanobacteria were obtained from the NCBI

Table 1 The primary protein structure of αPC from which a multiple alignment was generated via ClustalW2 (Larkin *et al.* 2007). A codon-equivalent multiple alignment (CEMA) was then manually generated in GeneDoc (Nicholas and Nicholas 1997) using the respective coding sequences (CDS) of the *cpcA* gene. These alignments were then used to design a single degeneracy primer set to detect the gene

Phycocyanin α-subunit (αPC/*cpcA*)		
Taxonomy	Accession (GI)	CDS
Acaryochloris marina MBIC11017	YP_001521631.1	gi|158340280:c148530-148042
Arthrospira maxima CS-328	ZP_03271568.1	gi|209522890:c153475-152987
Arthrospira platensis str. Paraca	ZP_06380686.1	gi|254349541:1530-2018
Crocosphaera watsonii WH 8501	ZP_00516609.1	gi|67923114:27350-27838
Cyanobium sp. PCC 7001	ZP_05045216.1	gi|254430111:1503852-1504340
Cyanothece sp. PCC 7424	YP_002375498.1	gi|218437013:c178991-178503
Cyanothece sp. PCC 7425	YP_002482426.1	gi|220905643:c1537801-1537313
Cyanothece sp. PCC 7822	YP_003886916.1	gi|307149945:c1808486-1807998
Cylindrospermopsis raciborskii CS-505	ZP_06308539.1	gi|282900552:43296-43784
Fischerella sp. JSC-11	ZP_08984589.1	gi|354565113:c350240-349752
Gloeobacter violaceus PCC 7421	NP_926164.1	gi|37519569:3425312-3425800
Lyngbya majuscula 3L	ZP_08428233.1	gi|332708240:c11382-10894
Microcoleus vaginatus FGP-2	ZP_08490947.1	gi|334116516:c429233-428745
Microcystis aeruginosa NIES-843	YP_001657460.1	gi|166362741:2210230-2210718
Nostoc azollae 0708	YP_003722228.1	gi|298489614:c3558921-3558433
Nostoc punctiforme PCC 73102	YP_001868554.1	gi|186680550:6544141-6544632
Raphidiopsis brookii D9	ZP_06304364.1	gi|282896246:102383-102871
Synechococcus elongatus PCC 6301	YP_171210.1	gi|56750010:c559913-559422
Synechococcus sp. PCC 7002	YP_001735446.1	gi|170076636:2301231-2301719
Synechocystis sp. PCC 6803	AAA91033.1	gb|U34930.1|SPU34930:856-1344
Thermosynechococcus elongatus BP-1	NP_682748.1	gi|22297544:2042263-2042751
Trichodesmium erythraeum IMS101	YP_724429.1	gi|113473942:c7709337-7708849

RefSeq database (Anon 2002) and are listed in Table 1. A multiple alignment on the αPC amino acid sequences was performed using ClustalW2 (Larkin *et al.* 2007) with Gonnet weighting matrices (Gonnet *et al.* 1992) to determine areas containing highly conserved residues. Global alignment was chosen because the αPC sequences were highly related. The complete *cpcA* ORF sequences were then manually aligned in GeneDoc (Nicholas and Nicholas 1997) to obtain a codon-equivalent multiple alignment (CEMA). For the initial primer design, Primer3 (Rozen and Skaletsky 2000) was used on the consensus sequence to obtain primers in locations of both high amino acid residue conservation and codon bias. The forward primer required redesign by inspection and included deoxyinosine-modified bases at positions of high degeneracy (Ohtsuka *et al.* 1985). Primer Blast (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) was then performed with this detection set as an input and no specified template against the nonredundant cyanobacteria (taxid: 1117) sequence library as a form of *in silico* PCR.

Next, 35 reference sequences of the 18S SSU gene of various fungi, green plants and cryptomonads were obtained and are listed in Table 2. These nucleotide sequences were then aligned in ClustalW2. The consensus sequence was then subjected to Primer Blast with cyanobacteria mispriming library and again for *in silico* PCR against Chlorophyta (taxid: 3041).

The primer sequences (Integrated DNA Technologies, Coralville, IA) used in *cpcA* detection were 5′-ATGAA AACCCCICTIACIGAAG (*cpcA*-F2) and 5′-ACCGTGGTT AGCTTTGATGT (*cpcA*-R1) with reported melting temperatures of 59·2 and 54·4°C, respectively. For eukaryote detection, 5′-TGTCAGAGGTGAAATTCTTGGA (18S rDNA-F1) and 5′-ACATCTAAGGGCATCACAGACC (18S rDNA-R1) oligos with melting temperatures of 53·9 and 56·6°C, respectively, were used. Primer-working solutions contained 10 μmol l^{-1} of each forward and reverse primer, further diluted in TE buffer (pH 8·0), for each set.

To test the *cpcA* detection capabilities across all organisms in this study, 50 μl reactions consisting of 1·25 U *Taq* polymerase (Invitrogen, Grand Island, NY), 20 mmol l^{-1} Tris–HCl (pH 8·4), 50 mmol l^{-1} KCl, 2·0 mmol l^{-1} $MgCl_2$, 0·2 mmol l^{-1} dNTPs (ea.), 0·5 μmol l^{-1} each primer and 50-ng DNA template were prepared in 200-μl polypropylene tubes. These reactions underwent one cycle of (94°C for 3:00), 32 cycles of (94°C for 0:45, 53°C for 0:30, 72°C for 0:45), one cycle of (72°C for 10:00) and a final incubation at 4°C in a Bio-Rad DNA Engine Peltier Thermal Cycler (model PTC0200; Bio-Rad, Hercules, CA).

The detection of the 18S SSU sequence had identical reaction composition, save the magnesium chloride concentration, which was 1·5 mmol l^{-1}. These reactions underwent one cycle of (94°C for 3:00), 30 cycles of (94°C for 0:45, 50°C for 0:30, 72°C for 0:45), one cycle of (72°C for 10:00) and a final incubation at 4°C.

As a positive control for the presence of PCR-quality DNA template in *E. coli*, the *gadA/B* detection primer set described by McDaniels *et al.* (1996) was used in four 50 μl reactions with 2·0 mmol l^{-1} $MgCl_2$. These reactions underwent one cycle of (94°C for 3:00), 30 cycles of (94°C for 0:45, 50°C for 0:30, 72°C for 0:45), one cycle of (72°C for 10:00) and a final incubation at 4°C. DNA extraction, amplification via PCR and gel electrophoresis were performed at least twice for each species/primer combination tested.

Table 2 Sequences obtained and used in the ClustalW2T (Larkin *et al.* 2007) multiple alignment of the 18S small subunit (SSU) ribosomal RNA gene, designed for the detection of eukaryotic DNA within a sample to help prevent false positives from cryptophyta and rhodophyta plastids

18S ribosomal RNA SSU	
Taxonomy	Accession (GI)
Actinastrum hantzschii	FM205884.1
Archaeospora leptoticha	AB047306.1
Basidiobolus haptosporus	AF368504.1
Cercosporella virgaureae	GU214658.1
Chlorella sorokiniana	FM205860.1
Chlorella sp. CB 2008/73	HQ111435.1
Chlorella vulgaris strain CCAP 211/11F	AY591515.1
Coronastrum ellipsoideum strain UTEX LB1382	GQ507370.1
Cryptophyceae sp. CCMP2293	GQ375265.1
Diacanthos belenophorus	AY323837.1
Dictyosphaerium sp. CB 2008/108	GQ507371.1
Dothistroma pini strain CBS 116487	GU214532.1
Endogone lactiflua isolate AFTOL-ID 45	DQ536471.1
Endogone pisiformis strain DAOM 233144	NG_017181.1
Hemiselmis virescens	AJ007284.1
Hindakia fallax strain CCAP 222/30	GQ487224.1
Hindakia tetrachotoma strain CCAP 222/78	GQ487240.1
Komma caudata	U53122.1
Lobosphaeropsis lobophora	FM205833.1
Marvania coccoides	FR865696.1
Meliniomyces variabilis strain UAMH 8861	AY762619.1
Micractinium pusillum	FM205873.1
Mortierellaceae sp. LN07-7-4	EU688964.1
Mycosphaerella graminicola strain CBS 115943	GU214540.1
Neochloris aquatica	FR865697.1
Passalora fulva strain STE-U 3688	AY251109.2
Proteomonas sulcata	AJ007285.1
Pseudocercosporella sp. CPC 10050	GU214685.1
Ramichloridium cerophilum strain CBS 103.59	EU041798.2
Rhizophlyctis rosea strain JEL 318	NG_017175.1
Rhodomonas sp. M1480	AJ007286.1
Saccharomyces cerevisiae	Z75578.1
Taphrina alni	AJ495831.1
Zasmidium anthuriicola strain CBS 118742	GU214595.1
Zygomycete sp. AM-2008a isolate 105	EU428770.1

PCR products were verified via agarose gel electrophoresis. Five microlitres of each reaction product and 1 μl of 6× Orange DNA Loading Dye (no. R0631; Fermentas, Glen Burnie, MD) were loaded into a 2% low EEO agarose (no. A1016; US Biological, Swampscott, MA) gel stained with 0·5 μg ml^{-1} ethidium bromide. An electric tension of 50 V was applied for 10 min to set samples in the gel and immediately followed by 100 V for 60 min in 0·5× TBE (Sambrook 2001). Visualization was performed with a UVP Bioanalyzer in conjunction with UVP VisionWorks LS (ver. 6.5.2) Acquisition and Analysis for product molecular weight and concentration estimation.

Results

The ClustalW2 multiple alignment of the accessed sequences for αPC (Table 1) showed high conservation with limited gaps (Fig. 1). Use of conserved areas allowed for the design of primers producing expected products of virtually equal length throughout these sequences (*Gloeobacter violaceus* being the only exception, with a 6 bp gap) whose 5′ termini locations are 0 and 423 with respect to $cpcA_{Syn6803}$. The amplified region includes the Cys_{84} phycocyanobilin-binding residue codon. Because single degeneracy was desired, some mismatching occurs within the annealing region. The mean number of mismatches over the 22 sequences was calculated (omitting modified bases) to be 1·45 for cpcA-F2, 2·50 for *cpcA*-R1 and 3·95 for the set. The multiple alignment and respective CEMA for the cpcA-F2 region for αPC/*cpcA* is shown in Fig. 1.

Even under lax conditions in Primer Blast, the 18S detection set showed no potential mispriming of expected length in cyanobacteria. The overwhelming majority of expected *in silico* products were 550–560 bp in length. The forward and reverse primer 5′ termini locations are 894 and 1450 with respect to the *S. cerevisiae* accession sequence.

Under more specific reaction conditions during preliminary testing, *cpcA* was detected in all cyanobacteria strains with the exception of the currently unsequenced *Plectonema* sp. Optimization was performed to maximize the amplification of *cpcA* product in this strain. The reaction products were subjected to typical gel quantification with the results depicted in Fig. 2. As expected, some mispriming did occur under low annealing temperature and high salt concentrations (indicated in Fig. 2 by *). Optimal conditions were used in all *cpcA* experiments and can be found in the materials and methods section or abbreviated in Table 3.

Electrophoresis results of the PCR products confirm *cpcA* detection in cyanobacteria strains, as observed in (lanes 1–5, Fig. 3, *cpcA*). No amplification was observed

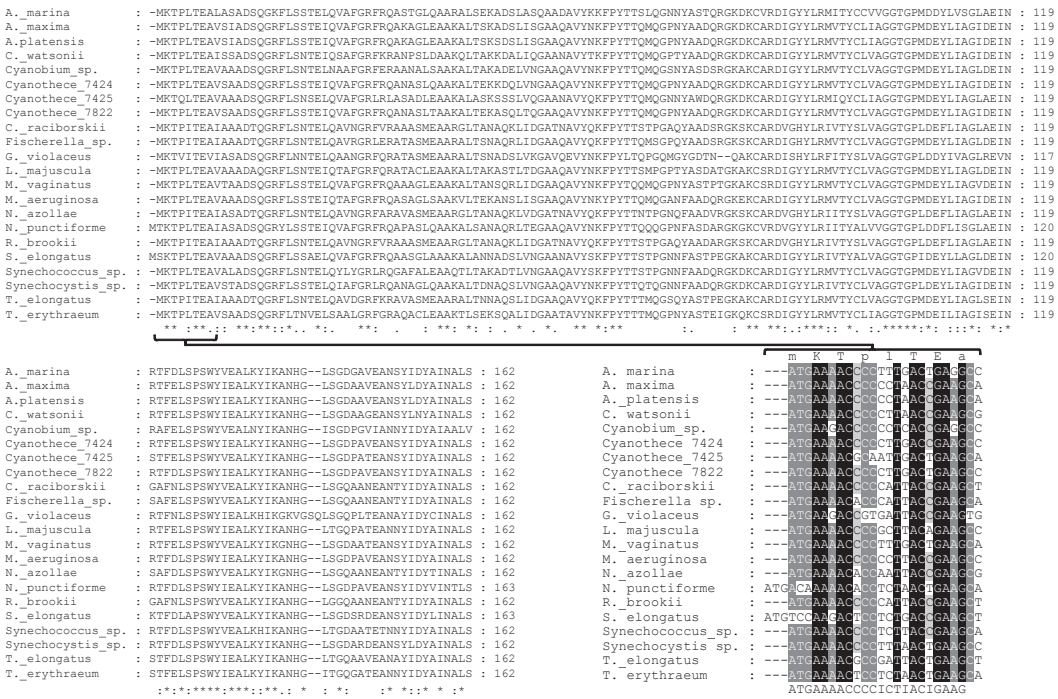

Figure 1 ClustalW2 multiple alignment of αPC from the cyanobacteria sequences obtained through NCBI and listed in Accession Table 1. A codon-equivalent multiple alignment (CEMA) for the *cpcA* gene was produced in GeneDoc (Nicholas and Nicholas 1997) to obtain regions of high codon bias. Primers were designed off of this CEMA in areas of high conservation. The *cpcA*-F2 (forward primer) region of the CEMA is shown (*cpcA*-F2 sequence is shown below) with respect to position of the αPC multiple alignment.

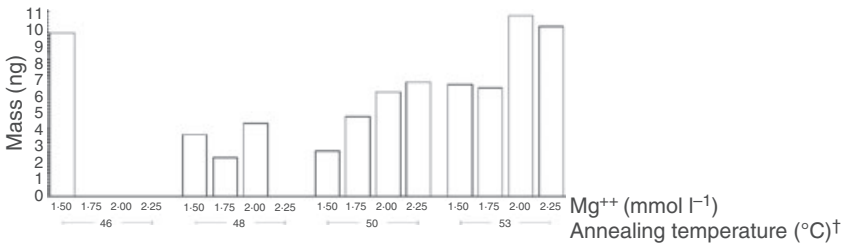

Figure 2 Resulting *cpcA* detection set PCR amplification products (8 μl from 50 μl reactions) from *Plectonema* sp. approximation of band mass from electrophoresis (2% agarose, 55 V, 60 min, 0·5× TBE) gel quantification. *Plectonema* sp. was chosen as optimization strain as it showed least efficiency for amplification. The other cyanobacteria tested in this paper showed specific amplification under a wide range of reaction conditions (n = 1). *Denotes non-negligible mispriming observed. †55 and 57°C were also tested under same Mg^{++} gradients, and no products were observed (not depicted).

Table 3 Sequences and properties of the primers designed and tested in PCR, with abbreviated reaction conditions utilized

Oligo properties					PCR conditions		
Sequence name	Bases	Sequence	GC content*	T_m (°C)*	T_{Anneal} (°C)	Mg^{++} (mmol l^{-1})	N_{cycles}
cpcA-F2	19	ATG AAA ACC CCI CTI ACI GAA G	40·9	59·2	53·0	2·00	32
cpcA-R1	20	ACC GTG GTT AGC TTT GAT GT	45·0	54·4			
18S rDNA-F1	22	TGT CAG AGG TGA AAT TCT TGG A	40·9	53·9	50·0	1·50	30
18S rDNA-R1	22	ACA TCT AAG GGC ATC ACA GAC C	50·0	56·6			

*Specifications provided by supplier (Integrated DNA Technologies), T_m at 50 mmol l^{-1} NaCl.

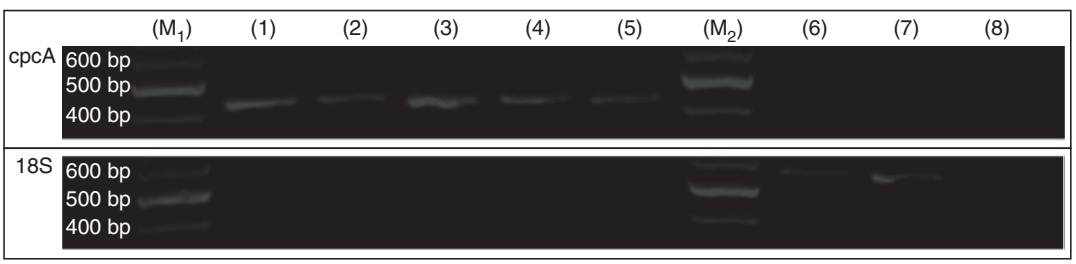

Figure 3 Agarose gel electrophoresis (2% standard agarose, 0·5 μg ml^{-1} ethidium bromide stain, 0·5× TBE) results of 5 μl of each PCR amplification products using the primer sets developed in this work. The first set (*cpcA*) detects the phycocyanin alpha-subunit-coding gene and the second set (18S) 18S ribosomal RNA coding sequence (n = 2). Lanes: (M) 5-μl 100-bp O'GeneRuler DNA ladder, (1) *Synechocystis* sp., (2) *Plectonema* sp., (3) *Nostoc muscorum*, (4) *Nostoc punctiforme*, (5) *Spirulina platensis*, (6) *Chlorella vulgaris*, (7) *Saccharomyces cerevisiae*, (8) *Escherichia coli**. *Escherichia coli* positive control performed in quadruplicate using *gadA/B* amplification primers (McDaniels *et al.* 1996). (data not shown)

in any noncyanobacteria, as observed in (lanes 6–8, Fig. 3, *cpcA*). Under these conditions, we estimated the amplified *cpcA* PCR products final concentrations to be between 9 and 20 ng μl^{-1} via gel quantification. The PCR results for the 18S detection showed no amplification in cyanobacteria (lanes 1–5, Fig. 3, 18S) and *E. coli* (lane 8, Fig. 3, 18S). Positive detection was observed in *S. cerevisiae* (lane 7, Fig. 3, 18S) and *C. vulgaris* (lane 6, Fig. 3, 18S) at the expected band lengths with final product concentrations of 10 and 5 ng μl^{-1}, respectively. Additionally, no other products were observed for these two strains. The *E. coli gadA/B* control showed positive results for quadruplicate reactions (data not shown).

Discussion

Cyanobacteria show much potential in driving down the production costs of many ecologically sound bio-products. However, because of the low yields currently observed from products produced by these micro-organisms improvements are needed before these processes can be considered economically sound. The difficulties associated with the high-throughput screening process make a robust check primer set prerequisite. Here, we have demonstrated the effectiveness of such a primer set.

Through the multiple alignment of a large population of cyanobacteria sequences, a detection primer set with

single degeneracy was shown to amplify the *cpcA* target sequence specifically in five different cyanobacteria from three different classes. Three cyanobacteria (*Synechocystis* sp., *N. punctiforme* and *S. platensis*) with known sequences for this gene were tested and successful detection was observed with as many as four mismatches in the set. Notably, the primers successfully amplified *N. punctiforme* in a single-step PCR, whereas previous universal check primer sets had failed (Neilan *et al.* 1995) or utilized semi-nested PCR (Rudi *et al.* 1997). This set has the potential to be a valuable tool in the future high-throughput screening of cyanobacteria that the high production bio-commodity field desperately requires. The *cpcA* detection primer set was demonstrated to be a robust tool for the detection of cyanobacterial DNA of adequate quality for routine molecular biology purposes. For axenic and unicyanobacterial cultures, the *cpcA* set alone is sufficient and recommended in regard to a DNA control. However, the two sets proposed in this paper combined possess a wider variety of uses than just high-throughput DNA control.

The result of $cpcA^+/18S^+$ could have multiple initial conditions because it only detects the presence of eukaryotic DNA, which could result in axenic algae cultures, mixed cultures of algae and cyanobacteria and cyanobacteria with nonalgae eukaryotic contamination (e.g. fungi). As a consequence, the primer sets proposed in this study could further be implemented in isolation of cyanobacteria from environmental samples containing higher algae and other various eukaryotes such as the many common fungal contaminants. Implementation of this isolation procedure would involve a combination of streak plating and colony PCR in conjunction with traditional microscopy. This could ensure with higher degree of confidence than microscopy alone that a colony lacks any eukaryotic organisms.

The *cpcA* controls could streamline many high-throughput methods involved in cyanobacteria research that will become more important in the near future as the interest in these unique organisms is consistently increasing. The current production capabilities of these organisms with respect to bio-industrial products of high value have yet to approach their production potential.

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