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An efficient DNA isolation protocol for filamentous cyanobacteria of the genus *Arthrospira*

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ABSTRACT

Thanks to their photosynthetic and nutritive properties, cyanobacteria of the *Arthrospira* genus are of interest as food supplements, as efficient oxygen producing life support system organisms for manned space flight, and for the production of biofuels. Despite these potential valuable applications, full genome sequences and genetic information in general on *Arthrospira* remain scarce. This is mainly due to the difficulty to extract sufficient high molecular weight nucleic acids from these filamentous cyanobacteria. In this article, an efficient and reproducible DNA extraction procedure for cyanobacteria of the genus *Arthrospira* was developed. The method is based on the combination of a soft mechanical lysis with enzymatic disruption of the cell wall. The comparison with other extraction protocols clearly indicates that this optimised method allows the recovery of a larger amount of DNA. Furthermore, the extracted DNA presents a high molecular weight, a reduced degradation and an excellent overall quality. It can be directly used for molecular biology purposes such as PCR, and clone library construction.

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1. Introduction

While DNA isolation seems to be a routine procedure for most organisms including viruses, bacteria, fungi, parasites, insects, mammals and plants (Liu, 2009), it is, for various reasons, a rather difficult one when performed on cyanobacteria (Fiore et al., 2000; Wu et al., 2000). The common problems encountered in DNA isolation from cyanobacteria mainly range from cell lysis efficiency (Billi et al., 1998 ; Fiore et al., 2000), to purification issues (Porter, 1988). Even though several methods for extracting cyanobacterial DNA have already been reported in literature (*e.g.* Mazur et al., 1980; T. Kallas et al., 1983), their respective efficiencies can greatly vary from one species to another (Fiore et al., 2000). The broad morphological, metabolic and ecological diversities found within the cyanobacterial phylum (Garcia-Pichel, 2000) might be the main reason for this inherent fluctuating efficiencies. Some species have proven to be particularly difficult to extract DNA from, requiring the development of specific DNA isolation protocols (Billi et al., 1998).

This is the case for members of the genus *Arthrospira Stizenberger. This genus* includes filamentous *cyanobacteria* with multicellular cylindrical trichomes arranged in a helicoidal shape, cross-walls visible by light microscopy, and containing gas-vesicles (Castenholz, 2001). *Arthrospira* are naturally growing in brackish water and saline lake environments of tropical and semi-tropical regions (Ciferri and Tiboni, 1985). In addition to being efficient oxygen producers, members of the *Arthrospira* genus appear to be rich in proteins, essential fatty acids, essential amino acids, vitamins and iron (Ciferri, 1983). "Generally recognised as safe" by the US Food and Drug Administration (Tarantino, 2003), *Arthrospira* are commonly sold as a nutrition supplements under the name "spirulina". Some medical studies claim that consumption of *Arthrospira* could have therapeutic properties as well, but no conclusive results could confirm its potential use as a drug so far (Ayehunie et al., 1998; Karkos et al., 2008). Due to its nutritive properties along with its photosynthetic way of life, *Arthrospira* sp. PCC 8005 has even been selected by the European Space Agency as a food complement and oxygen producer within the Micro-Ecological Life Support System Alternative, currently under development for long-term manned space missions (Hendrickx et al., 2006). Furthermore, certain species of *Arthrospira* have recently been investigated for the production of biomass as a precursor to hydrogen and liquid fuels (Ananyev et al., 2008).

Despite all these interests, genetic information on *Arthrospira* remains scarce. While the rapid development of genome sequencing and molecular biology has lead to remarkable discoveries in life sciences during the last decade, no sequencing projects have been successful in closing the genome of this cyanobacterium so far. During the sequencing of *Arthrospira* sp. PCC 8005 at the French

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National Sequencing Center, Genoscope (Evry, France), one of the major obstacles appeared to be the difficulty to obtain sufficient high quality DNA material from *Arthrospira* cultures (Janssen et al., in preparation). *Arthrospira* cells possess a very low amount of genomic DNA compared to other bacteria. The nucleic acids (DNA and RNA) represent *ca.* 4% of the dry weight of an *Arthrospira* cell, while it can reach up to 20% in fast-growing bacteria like *Bacillus subtilis* (Ciferri, 1983). Furthermore, *Arthrospira* cells are notably rich in substances which are difficult to remove from DNA extracts such as polysaccharides and polyphenols (De Philippis and Vincenzini, 1998). Finally, *Arthrospira* cells possess an important number of restriction endonucleases (Zhao et al., 2006). These enzymes are an important line of defence against the incorporation of foreign DNA, but also represent an additional difficulty for DNA extraction, as they can act as an autodestruction-like mechanism during the extraction process.

In this article, a novel DNA extraction procedure for filamentous cyanobacteria of the genus *Arthrospira* was developed. This method combines a dual mechanical and enzymatical lysis step, with a selective CTAB precipitation, to overcome the lysis and purity issues recurrently occurring with cyanobacteria (Billi et al., 1998; Fiore et al., 2000; Porter, 1988). The efficiency of the protocol was compared against a selection of DNA extraction methods: (i) The Wizard® Genomic DNA Purification System (Promega™), a universal bacterial DNA extraction method, based on enzymatic lysis and isolation of the nucleic acids on a silica membrane; (ii) the Miniprep protocol (Ausubel et al., 1995), a universal bacterial DNA extraction method, relying on enzymatic cell wall lysis in liquid cultures; (iii) a Fast-Prep®-based extraction procedure (Feurer et al., 2004), a universal extraction DNA procedure, relying on mechanical lysis of the cells by bead beating; (iv) a specific method for *Arthrospira* sp. (Baurain et al., 2002), relying on several washing steps to remove lipophilic pigments and render the samples more susceptible to enzymatic lysis; and (v) a procedure for the isolation of high molecular weight DNA from plant–fungus complexes (Möller et al., 1992), using a combination of both mechanical and enzymatic lysis. The high quantity, quality and suitability of the purified *Arthrospira* DNA for molecular PCR based reactions, and for clone library construction, were demonstrated.

2. Materials and methods

2.1. Strains and culture conditions

The protocol was originally optimised for the strain *Arthrospira* sp. PCC 8005. Additional tests were carried out on three strains. Strains PCC 8005, PCC 9108 and PCC 9223 were obtained from the Pasteur Culture Collection of Cyanobacteria. The *Arthrospira fusiformis* MOZ 1/2 strain was kindly provided by Dr. Krzysztof Waleron (University of Gdansk, Poland). All strains were cultivated in "Zarrouk" medium (Zarrouk, 1966) as 100 ml cultures in 250 ml Erlenmeyer flasks under normal ambient atmosphere. The cultures were kept in a light incubator at 6000 lx, at 30 °C, and shaken at 100 rpm (Braun Certomat BS-1 Shaking Incubator, Sartorius Stedim Biotech). Extraction samples were harvested at one third of the exponential growth phase (OD 750 nm ~1.0).

2.2. DNA extraction and purification

The following extraction procedures were repeated 4 times, each time on 4 different biological replicates, to assess the reproducibility of the methods. For each DNA extraction cells were collected by centrifugation of 5 ml from a stock culture (10 min, 3500 rpm) before being treated accordingly to each protocol. DNA samples were stored at −20 °C before further utilisation. Additional RNAse steps (1 µl of RNAse, 10 mg/ml for 1 h at 37 °C) were performed, whenever such steps were not included in the original protocols.

2.2.1. Wizard® genomic DNA purification system, Promega™

The extraction was performed according to the instruction of the manufacturer (http://www.promega.com/applications/dna_rna/productprofiles/a1120/default.htm).

2.2.2. Miniprep of bacterial genomic DNA (Ausubel et al., 1995)

Cells were resuspended in 567 µl TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) by repeated pipetting, and adjusted to a final volume of *ca.* 700 µl with 30 µl of 10% SDS and 3 µl of 20 mg/ml proteinase K. The mix was subsequently incubated for 1 h at 37 °C. Selective precipitation of proteins and polysaccharides was performed using 80 µl of CTAB/NaCl (10% CTAB, 0.7 M NaCl) in presence of 100 µl of 5 M NaCl. The samples were gently mixed by inversion and incubated for 10 min at 65 °C. Nucleic acids were thereafter isolated by a phenol:chloroform:isoamyl alcohol (25:24:1, Sigma-Aldrich®) separation, followed by one chloroform:isoamyl alcohol (24:1, Sigma-Aldrich®) separation. DNA was finally recovered by precipitation using 0.6 volume of isopropanol and centrifugation (5 min, 4 °C, 15,000 rpm). The DNA pellets were washed with 1 ml of cold 70% ethanol. Finally, the tubes were centrifuged one last time 5 min (4 °C, 15,000 rpm), the supernatant was discarded, and each pellet dried before being resuspended in 100 µl TE Buffer (10 mM Tris, 1 mM EDTA, pH 8.0).

2.2.3. Fast-Prep® DNA purification (Feurer et al., 2004)

Cells were resuspended into 200 µl TE buffer, and transferred into fresh 1.5 ml tubes. Samples were first pre-treated using lysozyme (50 µl of 5 mg/ml) and RNAse A (30 µl of 10 mg/ml), and the tubes were incubated for 30 min at 37 °C. The mix was transferred into 2 ml screw cap tubes (MO BIO Laboratories, Inc.) containing ~0.2 g of glass beads (212–300 µm, Sigma-Aldrich®), 30 µl 10% SDS and 200 µl phenol:chloroform:isoamyl alcohol (25:24:1, Sigma-Aldrich®). Mechanical lysis of the samples was subsequently performed: the tubes were placed in a Fast-Prep®-24 Instrument (MP™ Biomedicals), and mixed two times 40 s, force 6, with 1 min on ice between each run. The tubes were then centrifuged 5 min (4 °C, 15,000 rpm), and the supernatant collected. Purification of the DNA was performed by adding 0.1 volume from a 3 M ammonium acetate solution, along with 2.5 volumes of cold 100% ethanol. The tubes were placed 1 h at −20 °C, then centrifuged for 20 min (4 °C, 15,000 rpm). The supernatant was discarded, and the DNA pellets were washed with 1 ml of cold 70% ethanol, and dried before being resuspended in 100 µl TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0).

2.2.4. DNA extraction protocol for Arthrospira (Baurain et al., 2002; adapted from Pitcher et al., 1989)

Cells were washed one time with 100% ethanol to remove lipophilic pigments, and two times with 1 ml RS buffer (0.15 M NaCl, 0.01 M EDTA, pH 8.0). Cell lysis was done in presence of 100 µl of 50 mg/ml lysozyme for 30 min at 37 °C. Protein degradation was subsequently performed in presence of 2.5 µl of 10 mg/ml proteinase K, for 1 h at 37 °C. Cell lysis was achieved by a last incubation for 15 min at 37 °C, in presence of 500 µl GES buffer (60% wt/vol guanidium thiocyanate, 0.1 M EDTA, pH 8.0, and 1% wt/vol sarkosyl). The mix was kept for 10 min on ice, followed by an addition of 150 µl 5 M NaCl and 250 µl cold 7.5 M ammonium acetate. After the tubes were kept on ice for another 10 min, a first extraction was performed using 500 µl of phenol:chloroform:isoamyl alcohol (25:24:1, Sigma-Aldrich®), followed by a second one with 500 µl of chloroform: isoamyl alcohol (24:1, Sigma-Aldrich®). The nucleic acids were precipitated with 100% ethanol, collected by centrifugation, (4 °C, 15,000 rpm), and dried before being resuspended in 100 µl TE Buffer (10 mM Tris, 1 mM EDTA, pH 8.0). The samples were treated with 1 µl of RNAse (10 mg/ml) for 1 h at 37 °C.

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