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Evaluation of DNA extraction methods for freshwater eukaryotic microalgae

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ABSTRACT

The use of molecular methods to investigate microalgal communities of natural and engineered freshwater resources is in its infancy, with the majority of previous studies carried out by microscopy. Inefficient or differential DNA extraction of microalgal community members can lead to bias in downstream community analysis. Three commercially available DNA extraction kits have been tested on a range of pure culture freshwater algal species with diverse cell walls and mixed algal cultures taken from eutrophic waste stabilization ponds (WSP). DNA yield and quality were evaluated, along with DNA suitability for amplification of 18S rRNA gene fragments by polymerase chain reaction (PCR). QiagenDNeasy® Blood and Tissue kit (QBT), was found to give the highest DNA yields and quality. Denaturant Gradient Gel Electrophoresis (DGGE) was used to assess the diversity of communities from which DNA was extracted. No significant differences were found among kits when assessing diversity. QBT is recommended for use with WSP samples, a conclusion confirmed by further testing on communities from two tropical WSP systems. The fixation of microalgal samples with ethanol prior to DNA extraction was found to reduce yields as well as diversity and is not recommended.

1. Introduction

Scientific and commercial interest in microalgal communities has increased dramatically in the past five years with the realisation that such communities can be a globally valuable and sustainable source of biomass for the production of biofuels, fertilisers and animal feed. The coupling of microalgal production with wastewater treatment is important for the economic viability of such biomass production (Christenson and Sims, 2011).

Waste stabilization ponds (WSPs) are already used worldwide for low cost wastewater treatment in which microalgae play a crucial role in the assimilation of nitrogen compounds and production of oxygen for bacterial heterotrophic degradation of organic carbon (Camargo Valero and Mara, 2007; Ferrara and Avci, 1982; DiGiano, 1982; Senzia et al., 2002) to prevent eutrophication in receiving waters.

Despite the critical role of microalgae in the removal of nitrogen from wastewater, little is known about the composition of these communities. Microalgae are the key catalysts that control many of the processes in WSPs. An understanding of their ecology (i.e. the rules that govern their behaviour) could transform the design and operation of WSPs from its current simple empirical form to a more mature and predictive engineering practice (Curtis et al., 2003). Furthermore, the success of algal harvesting methods and treatment efficiency are also highly dependent on the algal community present (Christenson and Sims, 2011).

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The little that is known about the community composition of WSP systems is based on microscopy studies, such as those by El-Deeb Ghazy et al. (2008), Barthel et al. (2008), Shanthala et al. (2009) and Mara (1997). Microscopy though able to give an overview of the algal groups present is unsuitable when a large number of samples require processing as it; i) relies on the expertise of well trained personnel, ii) is limited to the presence of morphological markers (which many microalgal species lack), iii) is not accurate down to species level, and iv) is very time consuming.

Modern molecular methods can be used as an alternative to microscopy for assessing microalgal community structure. These techniques provide unequivocal identification of organisms based on evolutionary markers, as well as having a higher sample throughput. They have been applied extensively to study bacterial communities in diverse environments (van Elsas and Boersma, 2011; Sogin et al., 2006; Truu et al., 2009), to assess community dynamics in marine ecosystems (Larsen et al., 2001; Potvin and Lovejoy, 2009; Stoeck et al., 2007), and to study harmful algal blooms (Galluzzi et al., 2005; Vila et al., 2005; Tengs et al., 2001; Connell, 2002). In contrast, the study of microalgae within natural and engineered freshwater systems using molecular biology techniques is in its infancy. A few studies have been carried out on photoautotrophic picoplankton communities from lakes using fluorescence in situ hybridisation (FISH) (Lepere et al., 2010) and clone libraries, based on the 18S rRNA gene (See et al., 2005; Lefranc et al., 2005), though the majority of freshwater studies focus on cyanobacteria (Zwart et al., 2005; Ye et al., 2011). Very few studies have used molecular approaches to study microalgal communities in wastewater treatment. Moura et al. (2009), Yu and Mohn (2001) and Camargo Valero et al. (2009) focused on bacterial populations, while Furtado et al. (2009) isolated and cultured cyanobacteria, before using 16S rDNA gene sequencing to assess their identity. The only study thus far to have assessed eukaryotic microalgae in wastewater treatment plants (Ghosh and Love, 2011) detected greater species diversity than previously estimated by microscopy studies. Whilst this outcome is likely to be due to the increased resolution of molecular methods, it is imperative to consider possible sources of bias when using molecular techniques.

The DNA extraction method used on a sample can have a major impact on downstream community analysis. Eukaryotic microalgae have a large range of cell wall structures, which creates challenges for the unbiased, uniform and universal extraction of nucleic acids from such communities. Some microalgae have simple glycoprotein cell walls, while others contain decay resistant algaenans or silica compounds. It is therefore extremely important to identify DNA extraction methods that are affective for a broad range of cell types for total community DNA analysis.

Simonelli et al. (2009) tested eight protocols, including four commercially available kits on ten cultured marine microalgae to determine which protocol gave the best results in terms of DNA quantity and quality. They concluded that Qiagen Blood and Tissue (QBT) kit, Qiagen Plant Mini (QPM) kit and the Ultra Clean (UC) soil DNA isolation MoBio kit stood out as being the most effective in terms of extracting DNA that could be used to produce PCR products from a range of pure cultures. These three favoured kits have been used successfully in a number of mostly marine algal studies; QBT in Shi et al. (2009), Maloy et al. (2009) and Ghosh and Love (2011), QPM in Bowers et al. (2000), Dorigo et al. (2002) and Galluzzi et al. (2005) and UC in Simonelli et al. (2009), and Nejstgaard et al. (2008). While these methods are valid for marine samples they might not necessarily be applicable to freshwater eukaryotic microalgal communities due to inherent differences in community structure (and therefore cell wall types) and the levels of inhibitory substances common in WSP, such as humic acids (Amir et al., 2006), which have the potential to inhibit downstream processes such as PCR (Wilson, 1997).

In the current study we investigated the application of the three commercially available kits outlined above for the extraction of DNA from freshwater eukaryotic algae with a range of cell wall types, in both pure cultures and mixed natural consortia in WSP samples. DNA extraction was evaluated in terms of total DNA yield and purity, as well as the success in the amplification of targeted fragments of the 18S rRNA gene by PCR, and the diversity of dominant species in natural mixed cultures using denaturant gradient gel electrophoresis (DGGE). In addition, we investigated the effect of ethanol fixation on DNA extraction and subsequent PCR, as fixation is often used in the field to preserve cell morphology and community composition when samples cannot be immediately frozen. Ethanol is the simplest and safest fixative, which has previously yielded PCR products from some marine algae (Marin et al., 2001), in contrast to other common fixatives such as formalin and Lugol's solution, which in some cases have been shown to interfere with subsequent PCR reactions (Wilson, 1997; Marin et al., 2001; Godhe et al., 2002; Ahokas and Erkkila, 1993).

2. Materials and methods

2.1. Sample collection from WSP

Samples were collected from a WSP system that serves Larchfield community in Teesside, UK. The samples were collected from a cascade that feeds wastewater from one pond to another. 12 samples of 100 ml and 12 samples of 250 ml were collected and frozen at −20 °C on return to the laboratory. Another six 250 ml samples were collected. These samples were fixed with 250 ml of 98–100% ethanol in the field and then frozen at −20 °C on return to the laboratory.

Tropical samples were collected from two WSP systems in Fortaleza, Ceará, in the northeast of Brazil. One of the systems served the industrial district of the city, with a mixed influent, approximately 50% from industrial sources and 50% from domestic sources. The other system was fed purely domestic wastewater. Tropical samples were collected in the same way as UK samples from all of the ponds in both systems, though none of the samples were fixed with ethanol.

2.2. Sample preparation

Samples were defrosted and then centrifuged at 3392 × *g* (4200 rpm) for 2 h or 7690 × *g*, 10 min, which were shown to