



Metabolic adaptation of a *Chlamydomonas acidophila* strain isolated from acid mine drainage ponds with low eukaryotic diversity

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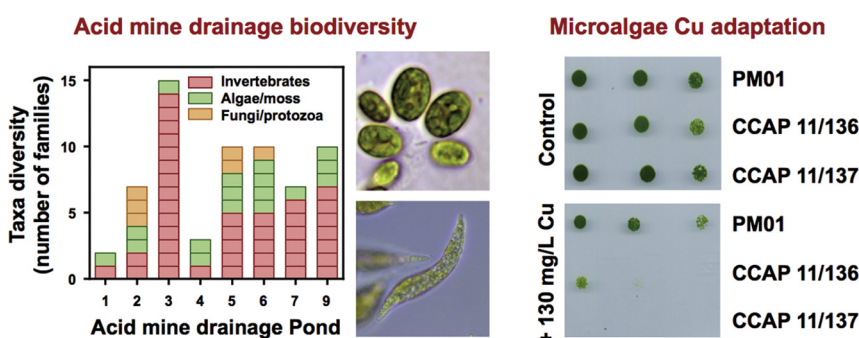
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HIGHLIGHTS

- Acid mine drainage ponds were examined for eukaryotic biodiversity.
- Biodiversity correlated with pH and metal concentration.
- A strain of *Chlamydomonas acidophila* had high tolerance to copper.
- This copper tolerance was much higher than other strains of the same species.
- The copper tolerance by the algae strain is partly due to metabolic adaptation.

GRAPHICAL ABSTRACT



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ABSTRACT

The diversity and biological characteristics of eukaryotic communities within acid mine drainage (AMD) sites is less well studied than for prokaryotic communities. Furthermore, for many eukaryotic extremophiles the potential mechanisms of adaptation are unclear. This study describes an evaluation of eight highly acidic (pH 1.6–3.1) and one moderately acidic (pH 5.6) metal-rich acid mine drainage ponds at a disused copper mine. The severity of AMD pollution on eukaryote biodiversity was examined, and while the most species-rich site was less acidic, biodiversity did not only correlate with pH but also with the concentration of dissolved and particulate metals. Acid-tolerant microalgae were present in all ponds, including the species *Chlamydomonas acidophila*, abundance of which was high in one very metal-rich and highly acidic (pH 1.6) pond, which had a particularly high PO₄-P concentration. The *C. acidophila* strain named PM01 had a broad-range pH tolerance and tolerance to high concentrations of Cd, Cu and Zn, with bioaccumulation of these metals within the cell. Comparison of metal tolerance between the isolated strain and other *C. acidophila* strains previously isolated from different acidic environments found that the new strain exhibited much higher Cu tolerance, suggesting adaptation by *C. acidophila* PM01 to excess Cu. An analysis of the metabolic profile of the strains in response to increasing concentrations of Cu suggests that this tolerance by PM01 is in part due to metabolic adaptation and changes in protein content and secondary structure.

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1. Introduction

Many freshwater bodies worldwide are highly acidic either due to natural causes or anthropogenic activities such as mining (Schultze, 2013; Smucker et al., 2014). Acid mine drainage (AMD) due principally to pyrite oxidation, is the cause of significant acidity in lakes and ponds situated in areas impacted by mining, and in rivers receiving mine water discharge (Johnson, 2003; Nordstrom, 2000). Because decreasing pH causes increased solubility of metals, AMD results in high concentrations of dissolved Fe, S and various trace metals such as Cu, Cd and Zn in the contaminated waters. Concentrations of nutrients, especially inorganic phosphate (PO₄-P), are also frequently very low (Nixdorf et al., 1998). The combination of toxic metals and nutrient limitation limits biodiversity and can cause significant ecosystem damage (Deneke, 2000; Smucker et al., 2014). Evaluation of the biological impacts of AMD allows quantification of pollution damage, allows understanding of fundamental processes of adaptation and can identify AMD-tolerant species that have biotechnological applications, such as bioremediation (Ñancuqueo and Johnson, 2011; Yun et al., 2014).

While prokaryotes in AMD environments have been extensively studied and reviewed (Johnson and Hallberg, 2003; Mendez-Garcia et al., 2015), there is still limited knowledge regarding the presence and roles of eukaryotes in these aquatic environments (Aguilera et al., 2006; Baker et al., 2004; Nixdorf et al., 1998). Photosynthetic microorganisms are found in many AMD ecosystems; however, the biodiversity of phytoplankton in such waters is severely limited and dominated by just a few acid-tolerant genera, such as *Chlamydomonas*, *Dunaliella*, *Euglena* and *Ochromonas* (Aguilera et al., 2006; Hargreaves et al., 1975; Ñancuqueo and Johnson, 2012; Nixdorf et al., 1998; Pedrozo et al., 2001). Despite being able to tolerate the highly acidic and metal-rich conditions, productivity of these extremophile microalgae is often limited by low inorganic carbon and nutrient availability in acidic waters (Beamud et al., 2007; Spijkerman et al., 2007b). A fairly broad diversity of heterotrophic fungi and protists has also been observed in acidic waters (Baker et al., 2004; Das et al., 2009), while the diversity and abundance of zooplankton is typically very low as most species are unable to tolerate these environments (Deneke, 2000).

The high concentrations of dissolved metals in AMD can cause toxicity to microorganisms through a wide variety of mechanisms, some of which are shared between metals and across different organisms, such as competition with essential metals, direct interactions with proteins and other molecules within the cell, and induction of oxidative stress (Sharma and Dietz, 2009). Metals such as Cu are particularly efficient at inducing the formation of reactive oxygen species (ROS) in contrast to non-redox active metals such as Zn and Cd (Valko et al., 2005). In most photosynthetic organisms, excess Cu has many detrimental effects with the photosynthetic apparatus, including direct inhibition of photosynthetic activity and degradation of chloroplast structures (Bernal et al., 2006; Küpper et al., 2003). Furthermore, non-extremophile microalgae exposed to high Cu conditions exhibit high concentrations of ROS and subsequent ROS-induced damage including lipid membrane peroxidation (Jamers et al., 2013; Jiang et al., 2016; Sabatini et al., 2009).

The adaptive mechanisms by which eukaryotic microorganisms including extremophile microalgae can survive in acid and metal rich conditions are still poorly researched but potential insights into these mechanisms are increasing. For example, proteomic approaches have indicated the importance of metal and acidity tolerance proteins, such as molecular chaperones of the Heat Shock Protein family (Cid et al., 2010; Gerloff-Elias et al., 2006). Likewise, genome sequencing and transcriptomics studies are beginning to identify the array of genes that might explain extremophile functional characteristics, some of which may have been obtained by horizontal gene transfer from bacteria. Genome sequences of the acidophiles *Chlamydomonas eustigma* (Hirooka et al., 2017) and *Galdieria sulphuraria* (Schönknecht et al., 2013) have recently been determined. Furthermore, transcriptomic approaches are beginning to provide insight into the molecular mechanisms of

Chlamydomonas acidophila tolerance in response to Cd and Cu exposure (Olsson et al., 2015; Puente-Sánchez et al., 2018), and *Dunaliella acidophila* in response to Cd (Olsson et al., 2017; Puente-Sánchez et al., 2016), although further experimental analyses of these transcriptomic datasets are needed.

AMD tolerant biota might have potential for bioremediation, with biological-based processes potentially more cost effective and sustainable than chemical based methods such as anoxic limestone drains and chemical addition (Geller and Schultze, 2013; Hedin et al., 2010; Johnson and Hallberg, 2005). Bioremediation methods can include utilisation of bacterial SO₄ reduction and neutralisation (Neculita et al., 2007) or aerobic wetlands that can oxidise and precipitate dissolved metals (Dean et al., 2013). However, eukaryotic algae that can tolerate AMD conditions may be an alternative bioremediation agent (Abinandan et al., 2018; Das et al., 2009). Novel extremophile algal strains that show high acid and metal tolerance, and metal bioaccumulation traits are therefore needed for such applications. In addition, extremophile algae may have other biotechnological applications, such as a source of novel high-value chemicals including nutritional vitamins and anti-oxidants, food additives, and biofuels (Varshney et al., 2015).

The aim of this study was to identify eukaryotes, especially extremophile microalgae, in a series of standing waters affected by AMD with the intention to characterise a strain of microalgae for evidence of AMD adaptation. Following a screen of eukaryotic biota within nine Cu-rich AMD ponds, an extremophile chlorophyte microalgal strain identified as *C. acidophila* was examined in detail due to its abundance and ubiquity across the site and its high tolerance to acidity and dissolved metal concentrations, especially to Cu.

2. Materials and methods

2.1. Study site

The site for this study is Parys Mountain, a disused Cu mine, in Anglesey North Wales, UK. The site has been mined for Cu from the Bronze Age, until mining activities ceased in the early 1900s (Dean et al., 2013). The area consists of large amounts of exposed spoil, with large pits and depressions that have filled with rainwater, and now retain large amounts of metal-rich and acidic water (Fig. 1). In addition, precipitation ponds and lagoons were constructed at the base of Parys Mountain, which were built in order to extract metals from the water as part of the mining process, and also contain large volumes of AMD polluted water (Younger and Potter, 2012). All the ponds are situated at close proximity within a similar geology with the rocks naturally rich in Cu, Pb and Zn. It is the only known example of Kuroko type volcanogenic massive sulfides in the UK, though the geology has been disturbed by many millennia of underground and surface mining activities (Younger and Potter, 2012).

Of the various mining ponds and lagoons at the Parys Mountain site, nine ponds were examined (Fig. 1). Ponds 1–4 are located at an elevated position on the spoil outcrop, with one of these (Pond 4) on the side of a steep incline of the now drained large opencast (Fig. 1). The remaining five larger ponds (Ponds 5–9) are the precipitation ponds and lagoons at the base of the Parys Mountain outcrop, each adjacent to agricultural land. Ponds 1–4 and 9 are shallow and <1 m depth and subject to rapid variation in depth due to seasonal evaporation and rainfall. Ponds 5–8 are deeper and are typically 2 m in depth. All ponds showed little variation in depth across each pond. Ponds 1, 2 and 4 had no vegetation in or surrounding them, whereas the other ponds had surrounding vegetation and marginal wetland plants.

2.2. Field site sampling

Sampling at the nine AMD ponds was carried out in 2013 to 2015, including a spring (February and March), summer (June) and autumn (October) sampling regime in 2015. Water chemistry samples were

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