<u>Experimental Isolation – *Desmodesmus armatus*</u>

Abstract

Algae are photosynthetic eukaryotes, and are ubiquitous in most of the worlds marine and freshwater environments. A study was conducted to isolate and identify algae from an environment using morphological and phylogenetic identification techniques. The algal species *Desmodesmus armatus* was successfully identified by DNA sequencing and a phylogenetic tree with closely related species was constructed.

Sampling

A freshwater sample was obtained on 11/11/2024 in Uppsala, Sweden at the position 59.850°N, 17.632°E. The small pond was first disturbed before being sampled in order to generate the greatest diversity of organisms from within the sample. The pond had leaves and twigs from nearby trees inside, and contained rocks with apparent green algal growth on the surface (Figure 1).

Figure 1: Algae sample location photo

Culture

The pond water sample was allowed to settle for 15 minutes, to allow any debris to collect at the bottom of the falcon tube. This also allows for the tube to simulate the water column differences in algal communities that may be found (Algae Corner, 2024). Small 1ml samples were pipetted from the top, middle and bottom the tube into the first columns in a 48-well plate. In order to obtain pure algal colony cultures from the sample, samples were serially diluted using different media that encourages growth of particular groups of algae. MWC is a generic algal growth medium, and Z8 is used to encourage growth of golden algae (chrysophytes). The media was first diluted as to not shock the algae when they are first introduced to it. Samples were mixed when added to the growth media, and the process repeated for each row to create a 1:10 dilution series. The well plate was then sealed with parafilm and incubated at 18C under a 12 hour light cycle for 2 weeks. After 1 week, wells were observed under microscope to look for pure cultures. The well F8, containing Z8 media and the greatest dilution, appeared to be a pure culture with many identical morphologically species present. This well was selected for further investigation.

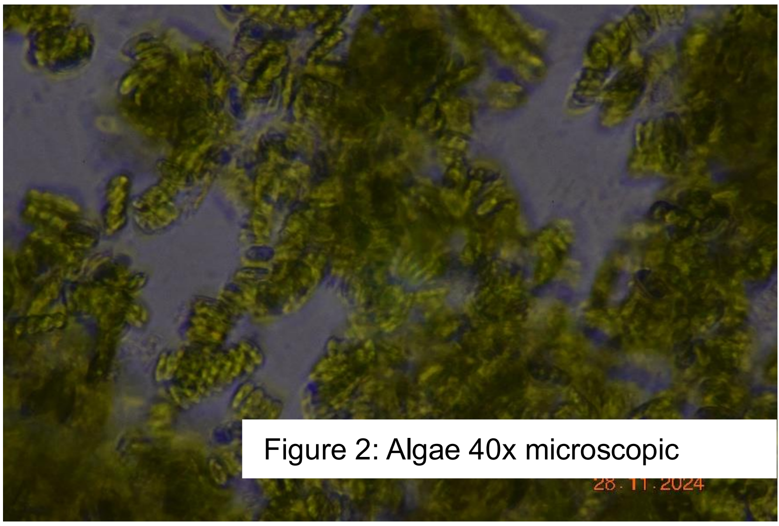

Figure 2: Algae 40x microscopic

Description

The algal species selected displayed several key characteristics later to used to confirm the species identification made was correct. The cells photographed (Figure 2) were green, rectangular in shape with rounded edges, and appeared to form stacks of four or more cells. The cells were non-motile, and based on these characteristics, a preliminary algal species determination was made. The group *Scenedesmus* appeared to have many of these traits (Bellinger, E.G. and Sigee, D.C., 2015). For example, they are commonly found in hypertrophic waters with many algae present, like the initial observation of excessive green growth in the pond. Many species within the group have cylindrical cells that join end to end in groups of two, four or more, much like the cells observed under the microscope. However, *Scenedesmus* are green alga (chlorophytes), and the growth media for the sample encourages growth of golden alga, and so further genetic analysis was required to confirm or refute this identification.

DNA sequencing and Phylogenetics

In order to confirm the algal species identification, DNA amplification and sequencing was performed. DNA was extracted from the pure culture, and amplified by PCR. Specific primers used for amplification were chosen, 3NDf as the forward primer and V4_euk_R1 as the reverse primer. These amplify the v4 region of the 18s small ribosomal subunit RNA gene, and this region is highly variable but conserved across eukaryotes, so is ideal for this application. PCR products were run on an agarose gel and produced a visible band, confirming the PCR was successful. The PCR product was then cleaned using ExoSap, forward and reverse primers added (separately) before being sent away for Sanger sequencing. This returns a chromatogram which was analysed for accuracy using SnapGene (2024). The sequences were trimmed for quality, and ambiguous bases were checked over. The reverse sequence was reverse complemented, and both sequences were aligned to create a consensus sequence. This was then used as a query with BLAST in NCBI (2024) to look for highly similar sequences, which was found to have 100% percentage identity to the species *Desmodesmus armatus.* Using the top 25 hits from BLAST, a reference database for algae and the assembled sequence, a phylogenetic tree was generated using aligned sequences via MAFFT (Katoh et al, 2002). This tree (Figure 3), generated using IQTREE (2024) and visualised with iTOL (2024), was used locate where the identified species falls and to explore environmental diversity. The constructed tree shows the assembled algae sequence falling with the *Desmodesmus* genus, with a bootstrap value of 100 supporting this conclusion. The branch connecting to the assembled sequence has a

Figure 3: Algae phylogenetic

bootstrap value of 0, but this could be caused by this sequence and the top 25 BLAST hits being too similar.

Desmodesmus armatus

The genus Desmodesmus belongs to the type species Scenedesmus (AlgaeBase, 2024), and so the earlier identification based on cell morphology was deemed accurate. They are commonly found in freshwater. Cells are elliptical in shape and found in four to eight cell clusters (Cao et al., 2020). Due to their fast growth rate and tolerance to environmental pollutants (Corcoran et al., 2018), they have been used an indicator organism for water quality.

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