

Lab 1 Report – *Monoraphidium griffithii*

Introduction :

The term algae is not an evolutionary coherent group, it contains photosynthetic organisms excluding land plants. They are the primary producers of aquatic environments where they are mainly found. They are of particular importance for life, as well as human activities as they produce approximately 50% of dioxygen in the atmosphere and a lot of valuable metabolites such as agar, pigments, proteins, lipids. Due to their taxonomic and evolutionary diversity, algae can be quite difficult to identify. The goal of this practical was to identify algae present in a water sample using morphological and sequencing techniques.

Sampling :

Water was collected at Ekebydammen (Figure 1), Uppsala, the 11th of November nearby the shores at position 59°50'41"N 17°35'50"E, in shallow water mixed with hydrophytes (Figure 2). Some Copepods were also captured while sampling.



Figure 1 : Ekebydammen



Figure 2 : Sampling point near the shore

Methods :

The algae were grown in two different culture medium, MWC or Z8. A dilution serie of the sample was performed in attempt to obtain pure culture. After 2 weeks of incubation at 18°C and 12 hours light cycle, the content of each pit was observed. Mixed colonies of diatoms, green algae were observed (Figure 3), but a pure culture from pit B3 was chosen for sequencing because of the purity of the culture and the large amount of visible cells (Figures 3 & 4).

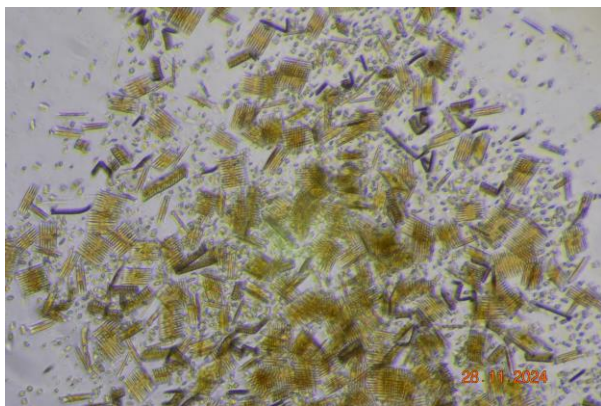


Figure 3: Diatoms from the genus *Fragilaria* coming from the collected sample

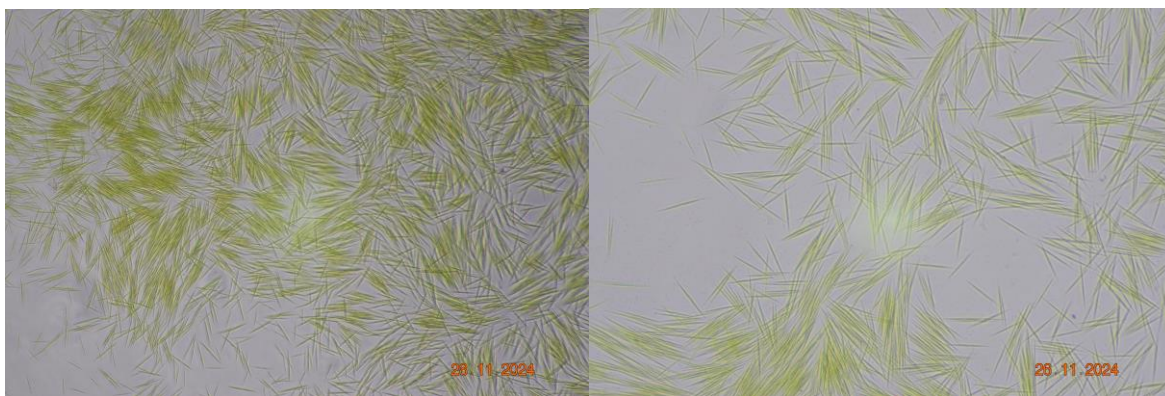


Figure 3 and 4 : Algae culture used for sequencing

A DNA extraction was performed by cracking-up three times the cell with immersion in liquid nitrogen. For the PCR reaction, the primers used were 3NDF and V4_euk_R1, amplifying the v4 region of the 18S rDNA. PCR products were separated on an agarose gel separating the fragments according to their size. However, no signal was observed for these fragments, indicating the failure of this operation. The reasons for this could be that the extraction phase did not allow to open the cells, or maybe that cells were tightened to the bottom of the pit and none were taken with the pipette. The identification phase had to be performed with a determination key only.

Identification :

The algae are naked, green, of needle like shape. The size is evaluated at 50µm approximately. (Figure 5). The cells are very abundant, gathered, forming dense bundles. With the determination key (Bellinger and Sigee), the species identified is *Monoraphidium griffithii*. There are several species in the genus *Monoraphidium*, among the species presented in the determination key, *M. arcuatum* and *M. contortum* could be candidates also but the curve of their body does not fit with the observed algae. An alternative could be *Ankistrodesmus sp.* but these species when present are gathered in more loose groups (Bellinger and Sigee).

The species : *Monoraphidium griffithii*

According to Bellinger and Sigeo, the genus *Monoraphidium* is well represented and very abundant in eutrophic ponds. It belongs to the clade Chlorophyta, more commonly called “Green algae”. Green algae have grown in interest for several years in bioremediation of heavy metals processes . Bácsi et al. have shown that *M. griffithii* could be used to remove zinc from water until 10 mg/L, opening the way to further research in the field of bioremediation and new techniques of depollution.

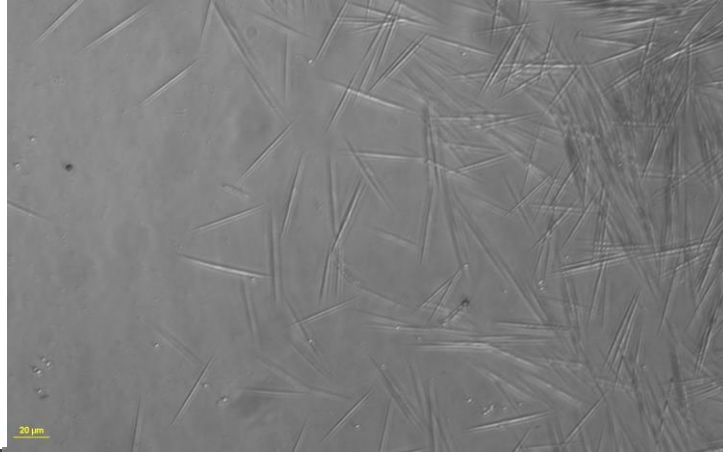


Figure 5 : *Monoraphidium griffithii* observed under the microscope

Resources :

Bellinger, Edward G., and David C. Sigeo. *Freshwater Algae : Identification, Enumeration and Use As Bioindicators*, John Wiley & Sons, Incorporated, 2015. *ProQuest Ebook Central*, <https://ebookcentral.proquest.com/lib/uu/detail.action?docID=1895748>.

Bácsi, I., et al. “The Sensitivity of Two *Monoraphidium* Species to Zinc: Their Possible Future Role in Bioremediation.” *International Journal of Environmental Science and Technology*, vol. 12, no. 8, July 2014, pp. 2455–66. <https://doi.org/10.1007/s13762-014-0647-3>.

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