ARPHA Preprints

PENSOFT

Methods

Author-formatted document posted on 03/06/2024

Published in a RIO article collection by decision of the collection editors.

DOI: https://doi.org/10.3897/arphapreprints.e128742

Steps of DNA extraction for molecular analysis of microalgae communities - a simple guide for beginners

Tena Radočaj, Oliver Barić, Serena Rasconi, Irena Vardić, Jurica Jug-Dujaković, Ana Gavrilović

ARPHA Preprints Author-formatted document posted on 03/06/2024. DOI: https://doi.org/10.3897/arphapreprints.e128742

Steps of DNA extraction for molecular analysis of microalgae communities - a simple guide for beginners

Tena Radočaj[1], Oliver Barić[1], Serena Rasconi[2], Irena Vardić[3], Jurica Jug-Dujaković[4], Ana Gavrilović[1]

[1]*University of Zagreb Faculty of Agriculture, Svetošimunska cesta 25, Zagreb, Croatia*
[2]*Université Savoie Mont Blanc, INRAE, CARRTEL, 74200 Thonon-les-Bains, France*
[3]*Ruđer Bošković Institute,Division for Marine and Environmental Research, P.O. Box 180, 10002 Zagreb, Croatia*
[4]*MJD Consulting, 21460 Stari Grad, Croatia*

Microalgae, including eukaryotic algae and cyanobacteria, form a diverse group of microscopic unicellular photosynthetic organisms that inhabit almost all ecological niches (Osanai et al., 2017). Their high growth rate, the simplicity of the cultivation process with minimal water consumption, the reduction of atmospheric CO_2, the possibility of year-round production in large outdoor facilities and photobioreactors as well as the low production costs are their main advantages compared to other raw materials (Fabris et al., 2020; Hussian et al., 2018). Apart from the advantages, one of the biggest challenges in this industry is the lack of reliable methods to detect and avoid biological contaminants, including parasites, which can affect cell growth and lead to a crash (Zhu et al., 2020). In recent years, the metabarcoding approach has been widely used to study the diversity and distribution of microalgae communities and is also a promising tool for contaminant detection in routine biomonitoring (Kezlya et al., 2023). This method consists of the following steps: DNA extraction, amplification and purification, quantification and collection (gel electrophoresis) and as a final step - library preparation and sequencing. The first step can be performed in any simple molecular laboratory using one of the available commercial kits, after which the properly extracted samples can be sent to a commercial company for NGS analyses. For successful extraction and further processing, it is necessary to collect the samples properly. For this reason, a possible detail of the procedure is presented here.

Collection and preparation of the samples

Samples of algal cultures should be collected from the culture vessel using a sterile pipette (single or micropipette with sterile tip). As commercial cultures are usually dense, 0.5 mL of sample should be sufficient. The pipetted sample is then placed in a 2 mL cryotube and absolute alcohol (1,5 mL) is added to the top of the tube. If DNA extraction is not to be performed immediately after this step, the samples should be stored at -80 °C until further analysis. If they need to be transported to the laboratory, liquid nitrogen or dry ice can be used.

For sampling from natural communities, Niskin samplers can be used to collect samples from the water column in sterile 1-litre bottles. Immediately after sampling, samples should be filtered in triplicate until filter saturation (up to 2 L per sample, depending on the density of natural population) through 0.2 µm pore filters (Whatman, Sigma Aldrich, UK) and frozen in liquid nitrogen until transport to the laboratory, where they are stored at -80 °C until processed for extraction (Kolda et al., 2020).

DNA extraction method for metabarcoding of different microalgae communities

Various commercial kits are used for this step according to the manufacturer's instructions. As this is a general procedure, these manufacturer's protocols are usually slightly modified for algae. As an example, DNA extraction for metabarcoding with the very commonly used Dneasy Power Soil Pro Kit (adapted for microalgae) is described below.

For DNA extraction, centrifuge the algae cultures for 20 minutes at maximum speed (Figure 1.) to obtain pellets. In the next step, the pellet is resuspended in 200 µl phosphate buffer, which is used for further DNA extraction steps using the Dneasy Power Soil Pro Kit (Qiagen, Germany) according to the manufacturer's instructions (Figure 2., Figure 3. and Figure 4.). Homogenisation is followed by an additional step of lysis by lysozyme (0.5 mg/mL) for 30 minutes at 37°C. The purity and concentration of the DNA should be further quantified, in our case with the Qubit 3.0 fluorimeter and with 1% agarose gel by gel electrophoresis (Figure 5.). After this step, the samples are ready to be sent for commercial NGS analyses.

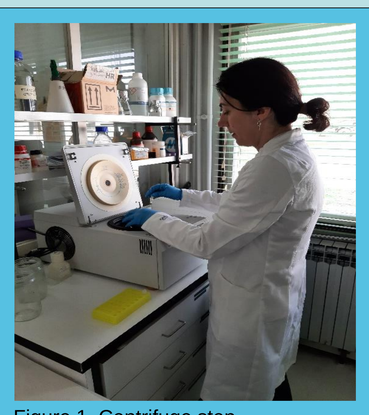

Figure 1. Centrifuge step

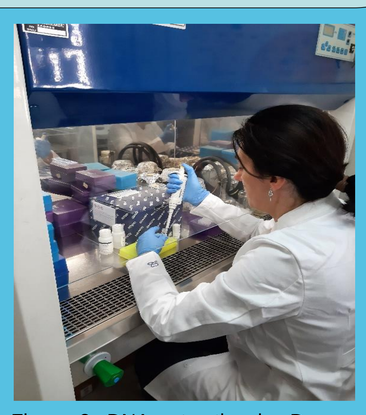

Figure 2. DNA extraction by Dneasy Power Soil Pro Kit (Qiagen)

Figure 3. Mixed zirconium bead tube for cells disruption

Figure 5. Electrophoresis in the agarose gel

REFERENCES

Fabris, M., Abbriano, R. M., Pernice, M., Sutherland, D. L., Commault, A. S., Hall, C. C., ... Ralph, P. J. (2020). Emerging technologies in algal biotechnology: toward the establishment of a sustainable, algae-based bioeconomy. Frontiers in plant science, 11, 279.

Hussian, M., Ellatif, A. (2018). The role of microalgae in renewable energy production: challenges and opportunities.

Kezlya, E., Tseplik, N., Kulikovskiy, M. (2023). Genetic Markers for Metabarcoding of Freshwater Microalgae. Biology, 12, 1038.

Kolda, A., Ljubešić, Z., Gavrilović, A., Jug-Dujaković, J., Pikelj, K., Kapetanović, D. (2020). Metabarcoding Cyanobacteria in coastal waters and sediment in central and southern Adriatic Sea. Acta botanica Croatica, 79 (2), 157-169.

Osanai, T., Park, Y. I., Nakamura, Y. (2017). biotechnology of microalgae, based on molecular biology and biochemistry of eukaryotic algae and cyanobacteria. Frontiers in Microbiology, 8, 118.

Zhu, Z., Jiang, J., Fa, Y. (2020). Overcoming the biological contamination in microalgae and cyanobacteria mass cultivations for photosynthetic biofuel production. Molecules, 25(22), 5220.

Acknowledgment

This publication is based upon work from COST Action 20125 - Applications for zoosporic parasites in aquatic systems (ParAqua), supported by COST (European Cooperation in Science and Technology). **Website**: www.cost.eu

cost EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY

Funded by the European Union

PARAQUA

DNA extraction steps using the Dneasy Power Soil Pro Kit (Qiagen, Germany) (17 steps):

1. *Add up to 250 mg of sample and 800 µl of Solution CD 1. Vortex briefly to mix.*
2. *Secure the PowerBead Pro Tube horizontally on a Vortex Adapter for 1.5-2 ml tubes, Vortex maximum speed for 10 min.*
3. *Centrifuge the PowerBead Pro Tube at 15,000 x g for 1 minute.*
4. *Transfer the supernatant to a clean 2 ml Microcentrifuge Tube.*
5. *Add 200 µl of Solution CD2 and vortex for 5 s.*
6. *Centrifuge at 15,000 x g for 1 min. Avoiding the pellet, transfer up to 700 µl of supernatant to a clean 2 ml Microcentrifuge Tube.*
7. *Add 600 µl of Solution CD3 and vortex for 5 s.*
8. *Load 650 µl of the lysate onto an MB Spin Column and centrifuge at 15,000 x g for 1 min.*
9. *Repeat step 8*
10. *Place the MB Spin Column into a clean 2 ml Collection Tube. Avoid splashing any flow-through onto the MB Spin Column.*
11. *Add 500 µl of Solution EA to the MB Spin Column, Centrifuge at 15,000 x g for 1 min.*
12. *Discard the flow-through and place the MB Spin Column back into the same 2 ml Collection Tube*
13. *Add 500 µl of Solution C5 to the MB Spin Column. Centrifuge at 15,000 x g for 1 min.*
14. *Discard the flow-through and place the MB Spin Column into a new 2 ml Collection Tube*
15. *Centrifuge at up to 16,000 x g for 2 min. Place the MB Spin Column into a new 1.5 Elution Tube.*
16. *Add 50-100 µl Solution C6 to the center of the white filter membrane.*
17. *Centrifuge at 15,000 x g for 1 min. The DNA is now ready for downstream applications.*

Figure 4. DNA extraction steps