Application note

Macroalgae DNA quantification and contaminant characterization with the NanoDrop One Spectrophotometer

Introduction

In coastal marine environments, macroalgae, or seaweed, serve as a shelter or food source for a variety of aquatic life.[1-2] Macroalgae also play a role in carbon sequestration, which involves capturing and removing carbon from the atmosphere to reduce the effects of climate change. Estuaries, oceans, and other bodies of water containing macroalgae act as biological carbon sinks that quickly sequester and store atmospheric carbon.[2-3] A key question of concern today is whether macroalgae can survive and adapt to rising sea temperatures and ocean acidification.

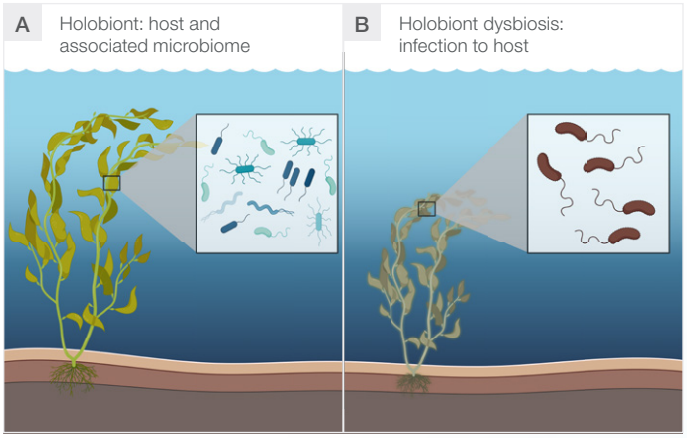

Figure 1. A) Holobiont consisting of seaweed and the associated microbiome in symbiosis. B) Dysbiosis of the holobiont causing an infection. Image created with BioRender.com.

The adaptability of macroalgae to the changing ocean environment depends largely on the seaweed-associated bacteria.[3-4] The host seaweed and the associated microbiome is called the holobiont, which describes the symbiosis as a functional unit (Figure 1A).[4-5] For example, bacteria depend on the macroalgae for polysaccharides as an energy source, while macroalgae depend on the bacteria for essential nutrients, such as vitamin B_{12}.[6-7] The seaweed-associated bacteria are also major players in seaweed morphogenesis.[4] When environmental conditions deviate from optimal, dysbiosis of the holobiont causes infections to the seaweed or atypical morphogenesis (Figure 1B).[4,8] However, the microbiome can adapt to protect the seaweed from the changing environment. Some marine bacteria, such as *Vibrio* spp., form a biofilm on the surface of macroalgae and serve as a protective barrier.[4]

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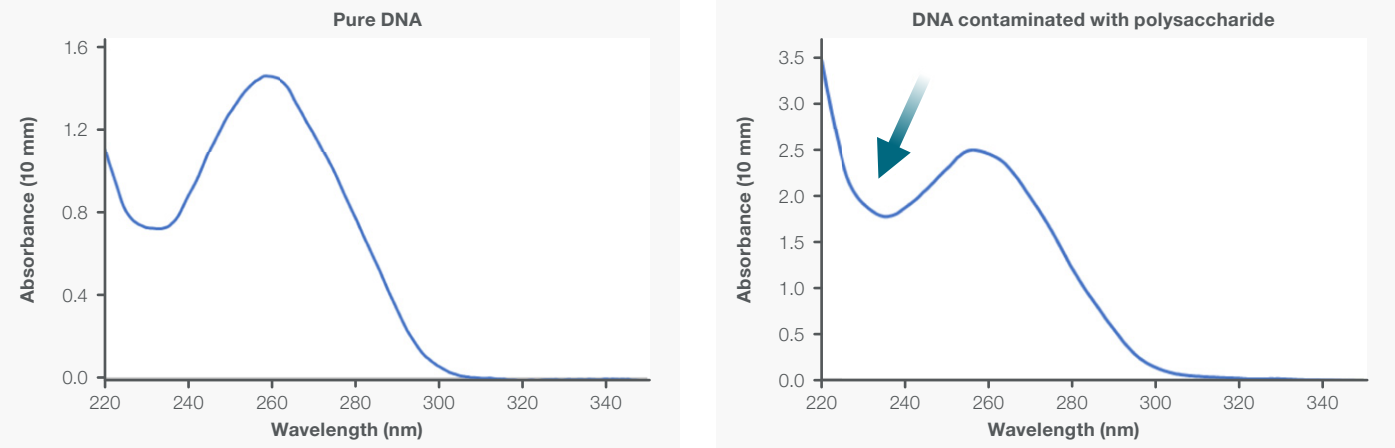

Figure 2. Polysaccharides and polyphenols absorb light at 230 nm, causing an absorbance increase in the typical nucleic acid trough. Image created with BioRender.com.

To better understand how macroalgae adapt to the changing environment, performing targeted qPCR or sequencing the genomes of bacteria associated with the holobiont can provide useful information. Polysaccharides and polyphenols are frequently co-extracted along with nucleic acids in macroalgae samples, some of which are known PCR inhibitors that inhibit the Taq polymerase or cross-link with the nucleic acid.[9-10] By applying a UV-Vis spectrophotometry checkpoint after extraction, the contamination can be visualized. An absorbance increase at 230 nm and a low A260/A230 purity ratio are indicative of polysaccharide or polyphenol contamination (Figure 2).[11] It is important to properly clean up the contamination to ensure a pure sample is used to prevent failed downstream reactions caused by inhibitors. This application note outlines extracting DNA from *Ulva* spp. and *Laminaria* spp. and performing a quality and quantity analysis with the Thermo Scientific™ NanoDrop™ One/One^{C} UV-Vis Microvolume Spectrophotometer in preparation for downstream qPCR or next-generation sequencing (NGS).

Experimental Procedures

Seaweed DNA and the associated bacterial DNA were isolated from *Ulva* spp. and *Laminaria* spp. (Carolina Biological Supply) using the Thermo Scientific™ MagMAX™ Plant DNA Isolation Kit (Cat. No. A32549), which is a magnetic bead-based purification kit optimized for nucleic acids with Invitrogen™ Dynabeads™ MyOne™ Carboxylic Acid beads. The nucleic acid extraction procedure was automated with the Thermo Scientific™ KingFisher™ Flex Purification System to enhance sample purity and to eliminate the hands-on requirement of tedious cetyltrimethylammonium bromide (CTAB) lysis and phenol, chloroform-based extraction procedures. Each extraction with the MagMAX isolation kit was performed per manufacturer's instructions, including the pre-processing steps, using 100 mg of plant tissue. Two samples of *Ulva* and *Laminaria* were not treated with RNase to illustrate the effect on the A260/A280 absorbance purity ratio. The *Laminaria* samples were treated with Polyvinylpyrrolidone, M.W. 40,000 (PVP40) (Thermo Scientific Chemicals, J62417.A1) to remove contaminating polysaccharides and polyphenols.

Additionally, one *Ulva* sample was extracted utilizing the CTAB (OPS Diagnostics, CEB 125-01) and phenol, chloroform-based method to compare purity results with the MagMAX isolation kit. After the extraction, DNA was quantified and qualified with the dsDNA application on the NanoDrop One spectrophotometer microvolume pedestal in replicates of 5 using a 2.0 µL sample volume for each replicate.

Sample	Concentration (ng/µL)	Standard Deviation (ng/µL)	A260/A280	A260/A230
***Ulva* with RNase**	47.02	0.38	1.88	1.24
***Ulva* without RNase**	290.68	1.14	2.15	2.45
***Ulva* CTAB extraction**	78.29	0.28	1.78	0.32
***Laminaria* with RNase**	53.22	0.36	1.95	1.81
***Laminaria* without RNase**	119.17	0.17	2.07	1.40

Table 1. DNA Concentration and purity results using the NanoDrop One spectrophotometer microvolume pedestal.

Results

The concentration and purity results of extracted dsDNA determined by the NanoDrop One spectrophotometer are shown in Table 1. The reported concentration standard deviations fall within the range of 0.17 – 1.14 ng/µL, detailing the reliability and accuracy of the NanoDrop One instrument in determining concentration.

The *Ulva* and *Laminaria* samples that were not treated with RNase have an inflated concentration in comparison to samples that were treated with RNase. Since the A260/A280 purity ratio of "pure" dsDNA is expected to be around 1.8 and around 2.0 for "pure" RNA, the higher-than-expected A260/A280 ratio is associated with RNA contamination in a dsDNA sample. This is due to the higher A260/A280 ratio of uracil in RNA (4.00) compared to thymine's lower ratio in DNA (1.47).[12] For successful qPCR or NGS, the dsDNA concentration must be accurate. The presence of RNA co-absorbing with dsDNA artificially increases the reported concentration and the sample is thus mistakenly overdiluted, affecting the downstream qPCR or NGS results. To combat the high A260/A280 ratio, adding RNase reduced the *Ulva* and *Laminaria* purity ratios from 2.15 to 1.88 and 2.07 to 1.95, respectively (Figure 3).

The generally accepted range for the A260/A230 purity ratio of "pure" dsDNA and RNA is 2.0-2.2. However, the lower cutoff is flexible depending on the downstream dilution factor and nature of the contaminant.[13] The *Ulva* dsDNA sample that was extracted using the CTAB-based method exhibited the lowest A260/A230 ratio of 0.32. The high viscosity of the sample and the absorbance increase at 230 nm represents polysaccharide contamination, which is detrimental to NGS library preparation and qPCR.[14] Figure 4 represents the purity improvement using the MagMax isolation kit as opposed to the traditional CTAB method. The addition of high-salt buffers and PVP40 aids in preventing co-precipitation of polyphenols and polysaccharides, as shown in the samples extracted using the MagMAX isolation kit (*Ulva* with/without RNase and *Laminaria* with/without RNase) with A260/A230 ratios ranging from 1.24 – 2.45.

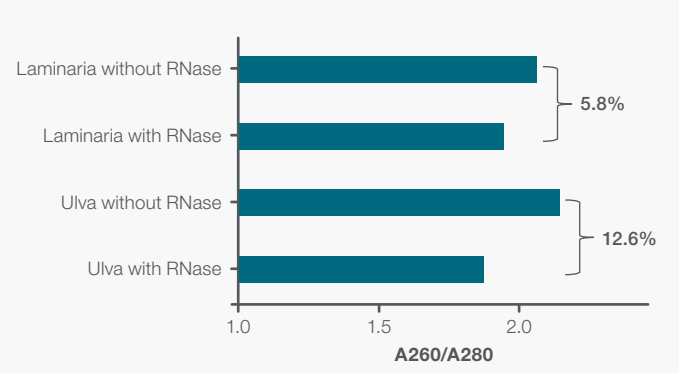

Figure 3. Observed increase in A260/A280 for DNA samples not treated with RNase. Percentage indicates percent change.

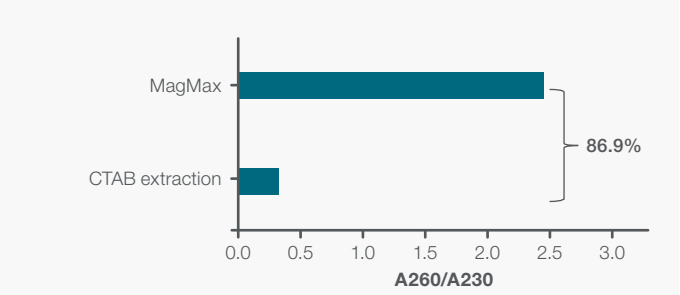

Figure 4. Purity improvement of A260/A230 using the MagMax isolation kit compared to the CTAB-based extraction method. Percentage indicates percent change.

Conclusions

Given the environmental impact of macroalgae, it is important to protect and maintain its health and longevity to ensure future carbon capture and habitat conservation. To obtain high-quality data from NGS or qPCR, the macroalgae dsDNA starting material must have a reliably determined concentration and purity. When extracting dsDNA from macroalgae, RNA, polyphenols, and polysaccharides are frequently co-extracted. Utilizing the MagMAX isolation kit along with RNase and PVP40 has been shown to reduce or remove the contaminating material compared to the CTAB and solvent-based extraction. The NanoDrop One spectrophotometer serves as a reliable tool for ensuring successful downstream assessments of the seaweed-associated microbiome and the adaptability to the changing ocean environment.

References

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