

Protein extraction from tough marine phytoplankton

Environmental Proteomics, Sackville, NB, Canada

CONTEXT

We extract total proteins from tough, recalcitrant marine phytoplankton for quantitative analyses of protein composition. We are seeking means to increase the throughput and reliability of our extractions. In this experiment, we compare our standard microprobe sonication/flash freeze protocol to extract total proteins from a single sample at a time, to the Precellys bead-based system to extract total proteins from multiple samples in parallel.[1]

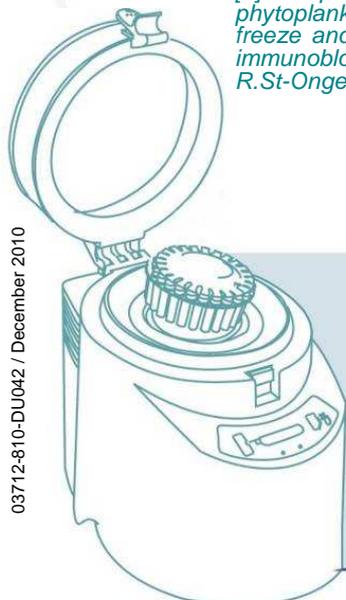
MATERIAL

- Precellys®24 homogenizer.
- Precellys® kit: 03961-1-004 (0.5mm glass beads).
- Samples: culture pellets from *Ostreococcus tauri*, *Thalassiosira pseudonana*, *Synechococcus sp.* WH7803, *Prochlorococcus marinus* PCC 9511 and *Synechococcus sp.* WH8101 re-suspended in buffer.
- Buffer: Protein extraction buffer (Agrisera, Cat. AS08 300) supplemented with the protease inhibitor 4-(2-aminoethyl)-benzene-sulfonyl fluoride HCl (417µM).

PROTOCOL

- Precellys®24: 400µl of suspension in 2mL Precellys tube / 6500 rpm, 3x20 sec (5 sec break) with Cryolys.
- Sonication/flash freeze: 400µl of suspension flash-frozen in liquid nitrogen, thawed and sonicated for 15 sec (amplitude: 30%). This was repeated once.
- Total protein quantification, Immunoblotting with relevant antibodies.

[1] Comparison of protein extraction efficacy from phytoplankton using microprobe sonication/flash freeze and PreCelllys bead beating protocols for immunoblotting. D.Campbell, A.Cockshutt and R.St-Onge.



RESULTS

The total yield of proteins extracted from each species was similar using the sonication/flash freeze protocol or the Precellys® bead beating systems (Table 1). Simultaneous, parallel extractions with the Precellys® system mean sample throughput and reproducibility are higher than with our sonication/flash freeze protocol. The immunoblots show that the quality of the protein extractions was comparable for both a large, soluble protein (ribulose-1,5-bisphosphate carboxylase oxygenase; Figure 1) and for a highly hydrophobic membrane protein (PsbA, D1) (not shown).

Table 1:

Organism	Concentration of proteins (µg µL ⁻¹)	
	Sonication/flash freeze	PreCelllys 0.5-mm glass beads
<i>Ostreococcus tauri</i>	1.20	1.05
<i>Thalassiosira pseudonana</i>	1.26	1.05
<i>Synechococcus sp.</i> WH7803	3.50	4.44
<i>Prochlorococcus marinus</i> PCC 9511	3.38	5.76
<i>Synechococcus sp.</i> WH8101	5.26	6.02

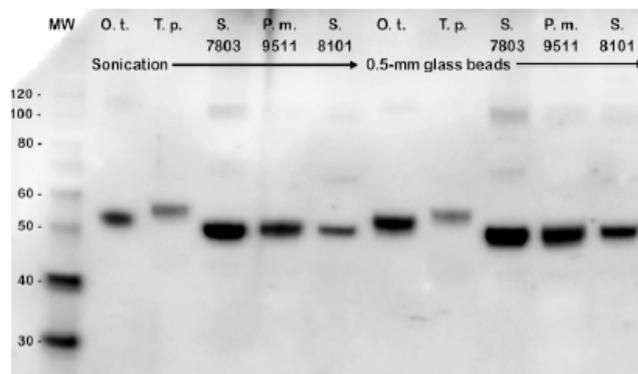


Figure 1: Comparison of ribulose-1,5-bisphosphate carboxylase oxygenase (RbcL) immunodetection from phytoplankton after protein extraction through sonication or Precellys bead beating. RbcL was detected by immunoblotting using a chicken anti-RbcL primary antibody and a horseradish peroxidase-conjugated goat anti-chicken secondary antibody. MW, molecular weight protein ladder (in kDa).

CONCLUSION

The Precellys® generated total protein extracts with yields and quality comparable to those of extracts obtained using probe sonication and flash freezing. The extractions with the Precellys® greatly increase throughput and improve reproducibility across samples.

Precellys® homogenizer is effective for the protein extraction from tough, recalcitrant marine phytoplankton.

For more details, please contact precellys@bertin.fr

Problem



Solution

