Protein extraction from nematodes using the SPEX Freezer/Mill

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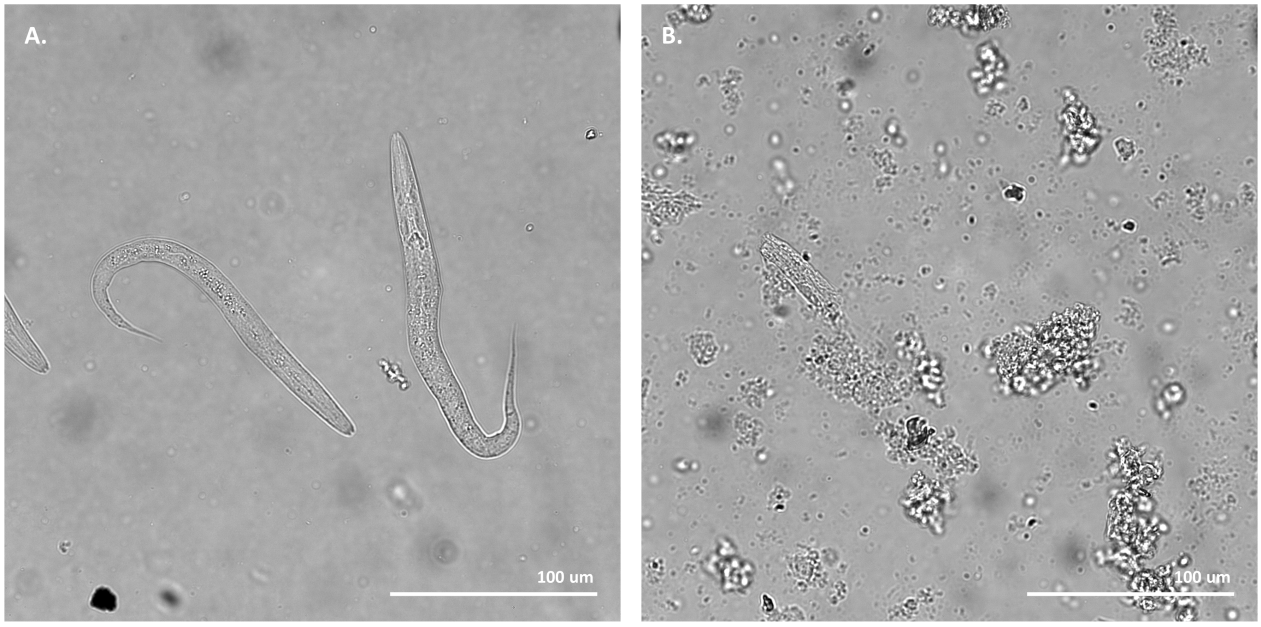
Introduction

Nematodes, like many other species, have an exoskeleton made of rigid chitin. This impairs protein extraction using classical methods (e.g. detergent-based such as RIPA) as the skeleton needs to be broken up mechanically. Several options exist, ranging from freeze-thawing the sample to bead beating. However, these solutions have the major drawback that the sample has to be maintained above freezing point and might get heated up due to the impacts of the beads, leading to protein degradation. We tested whether protein extraction could be carried out at liquid nitrogen temperature using the SPEX Freezer/Mill. For this, we used starved first larval stage animals (Picture 1A), for which the extraction has been known to be notoriously difficult as the animals are small and very tough to break apart.



Picture 1A: First larval stage animals after 12h starvation in liquid culture; 1B: First larval stage animal remnants recovered from melted cryogenically milled samples.

Protein extraction method

Animals were first recovered from their liquid growth media (S-basal) using centrifugation (1500g, 1'30'' in 15 ml conical tube). Hundred microlitres packed animals were then diluted with 600 ul PBS pH 7.4 and the mixture was pipetted dropwise into liquid nitrogen. The frozen droplets were then used for SPEX Freezer/Mill cryogenic milling.

Using 3 cycles of one minute shaking at speed 10, followed by 2 minutes cooldown with an initial precool of 5 minutes, we obtained a very fine powder in which no entire animal could be observed (Picture 1B).

After milling, all materials and tools were kept cooled in liquid nitrogen. The vial was removed, opened and powdered animals transferred into a precooled 50 ml Falcon conical tube, paying attention not to allow thawing of the sample powder. Out of 700 ul sample, 2.5 ml powder could be recovered.

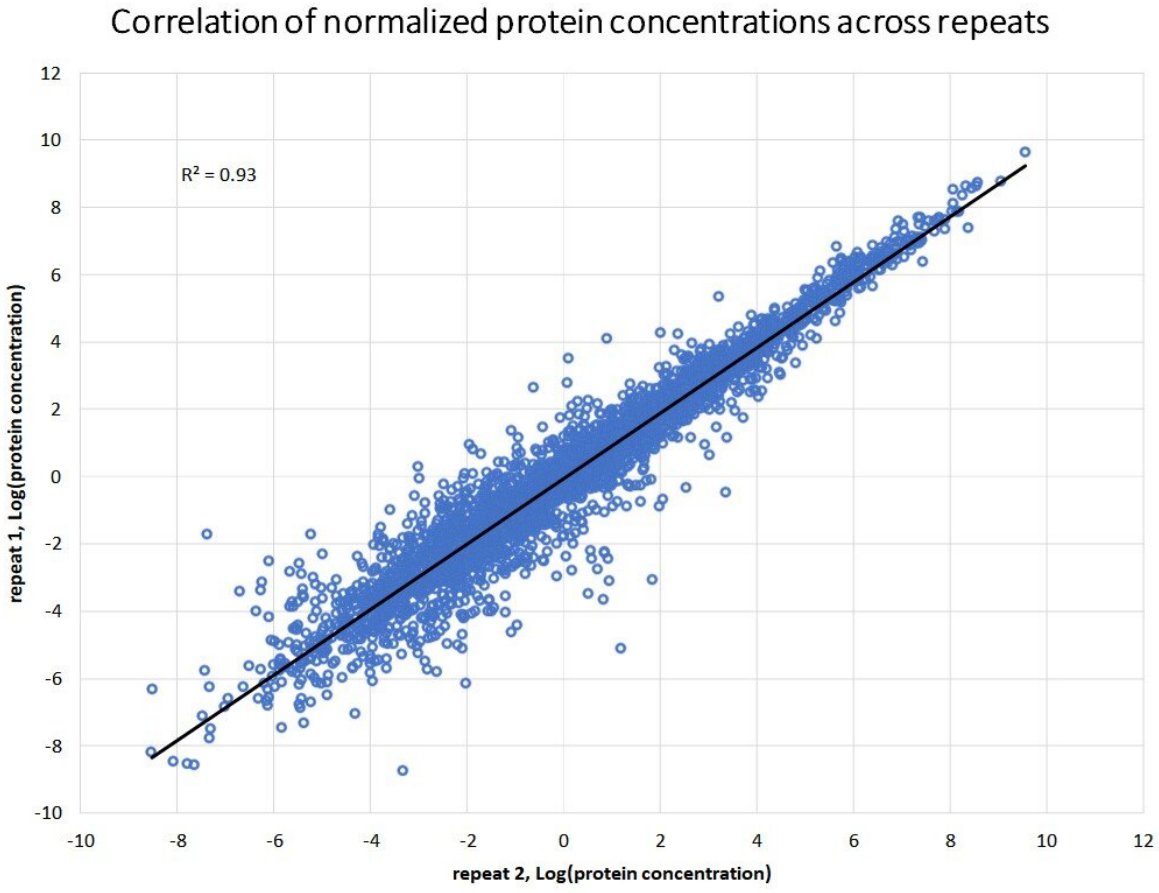


Figure 2: Correlation of protein concentrations from two repeats of extraction from C. elegans larva using SPEX Freezer/Mill. Circles are individual proteins quantified by unlabeled mass spectrometry. Black line is linear fit (R2=0,93).

Results

To determine the protein extraction efficiency, 70 ul of sample powder was melted. The protein concentration in the resulting liquid was 330 ug/ml, as measured by micro BCA protein assay kit (Thermo Scientific). The total amount of protein in each sample was thereby approximately 230 ug. Unlabeled mass spectrometry further showed good consistency in sample powder protein composition between replicate samples (Figure 2).