Metaproteomic comparison of cryoconite communities from Caucasian and Novaya Zemlya glaciers

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Supplementary materials 1

Detailed description of the proteomics analysis

Protein extraction

The scheme of protein extraction procedures is represented in figure 1. The frizzed samples were transferred to the laboratory and 1g of each sample were transferred to protein LoBind Eppendorf tube (2 ml) and mixed with 1 ml of 1% SDS solution. The samples were homogenized in mixer mill Retsch MM 400 for 25 min at 30 Hz. Then the samples were incubated in ultrasonic bath for 10 min at 80°C and were centrifugated at 15000 g for 15 min. The samples were transferred to a new tube and centrifuged again until there was no visible pellet.

The protein was precipitated from the SDS solution by four volumes of cold acetone with followed by incubation in -20°C or one hour and centrifugation at 15000 g for 15 min with 4°C. Protein pellet was washed by cold acetone and air dried for 5-10 min. Then it was resuspended in approximate six volumes of resuspension buffer (8M urea/50mM ammonium bicarbonate; Sigma). The protein concentration was measured by Qubit 4 fluorometer (Invitrogen) by QuDye Protein Quantification Kit (Lumiprobe). 10 ug of each sample was used for further tryptic digestion.

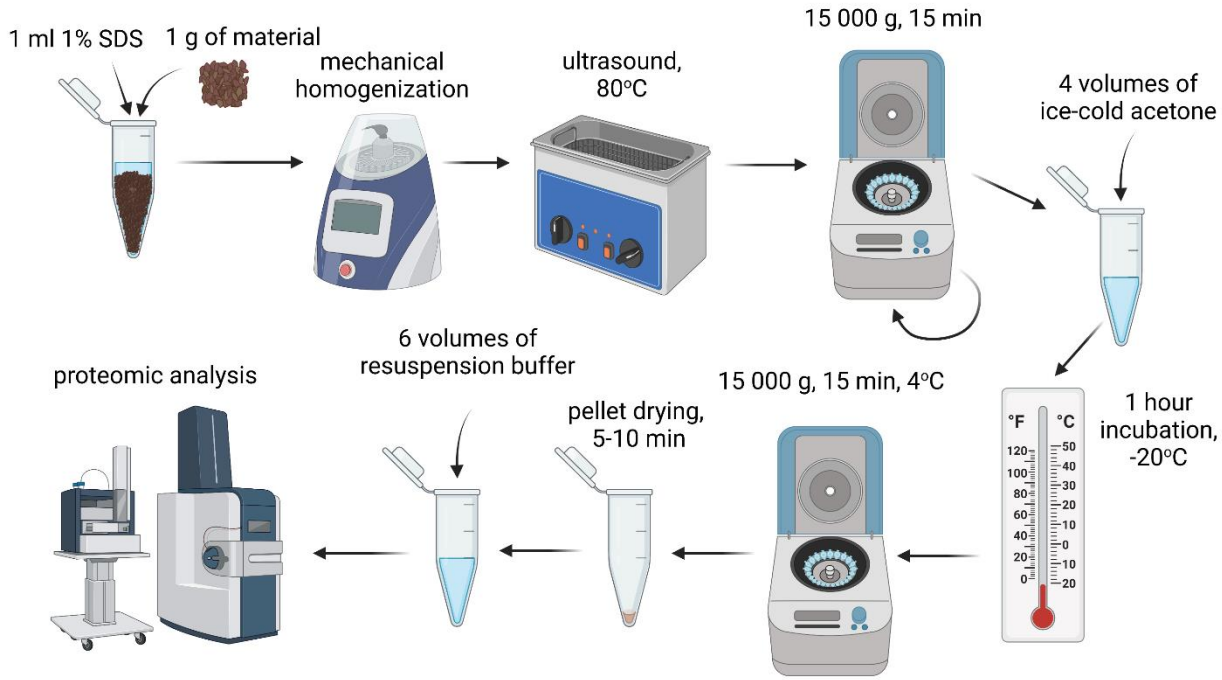

Figure 1. Schematic representation of protein extraction procedures. See the description above.

In-solution digestion

Tryptic digestion was performed by standard “in-solution” procedure. The samples were incubated for 1 hour at 37°C with 5 mM DTT (Sigma Aldrich) with subsequent incubation in 15 mM iodoacetamide for 30 minutes in the dark at RT (Sigma Aldrich,). Next, the samples were diluted with seven volumes of 50 mM ammonium bicarbonate and incubated for 16 hours at 37°C with 200 ng of Trypsin Gold (ratio 1:50; Promega, Madison, WI, USA). Then the samples were mixed with formic acid (Sigma Aldrich) to 1% final concentration, evaporated in Labconco Centrivap Centrifugal Concentrator, desalted with C18 ZipTip (Millipore) according to manufacturer recommendations and evaporated in Labconco Centrivap Centrifugal Concentrator. Desalted peptides were dissolved in 15 ul of water/0.1% formic acid for further LC-MS/MS analysis.

LC-MS/MS analysis

Shotgun proteomics analysis was performed in nanoLC-MS/MS with trapped ion mobility spectrometry on Bruker TimsToF Pro instrument.

HPLC was performed in one-column separation mode with Bruker Ten separation column (C18 ReproSil AQ, 100x0.75 mm, 1.9 μm, 120 A; Bruker Daltonics) in gradient mode with 500 nl/min flow rate. Phase A was water/0.1% formic acid, phase B was acetonitrile/0.1% formic acid. The gradient was from 5% to 30% phase B for 17,8 minutes, then to 95% of phase B to 18,3 minute with subsequent wash with 95% phase B up to 20.7 minute. The column was equilibrated with 4 column volumes before each sample.

CaptiveSpray ion source was used for electrospray ionization with 1600 V of capillary voltage, 3 l/min N2 flow and 180°C source temperature. The mass spectrometry acquisition was performed in automatic DDA PASEF mode with 0.5s cycle in positive polarity with the fragmentation of ions with at least two charges in m/z range from 100 to 1700 and ion mobility range from 0.85 to 1.30 1/K0.

Protein data analysis

Proteins were identified in the Peaks X Pro software (license on St. Petersburg State University) against the UniProtKB (SwisProt + TrEMBL) database (uploaded 01.05.21, number of sequences 214967504) and common Repository of Adventitious Proteins (cRAP; version 2012.01.01) as a contamination database. Only protein groups with at least two unique peptides and FDR < 1% were included for further data analysis in R. For Novaya Zemlya and Caucasus comparison we used proteins which were represented in at least two replicates of each glacier. Then we performed analysis of phyla distribution and functional annotation by Gene Ontology database.