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Evaluation of sodium deoxycholate as solubilization buffer for oil palm proteomics analysis V.2



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ABSTRACT

Protein solubility is a critical prerequisite to any proteomics analysis. Combination of urea/thiourea and 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) have been routinely used to enhance protein solubilization for oil palm proteomics studies in recent years. The goals of these proteomics analysis are essentially to complement the knowledge regarding the regulation networks and mechanisms of the oil palm fatty acid biosynthesis. Through omics integration, the information is able to build a regulatory model to support efforts in improving the economic value and sustainability of palm oil in the global oil and vegetable market. Our study evaluated the utilization of sodium deoxycholate as an alternative solubilization buffer/additive to urea/thiourea and CHAPS. Efficiency of urea/thiourea/CHAPS, urea/CHAPS, urea/sodium deoxycholate and sodium deoxycholate buffers in solubilizing the oil palm (*Elaeis guineensis* var. Tenera) mesocarp proteins were compared. Based on the protein yields and electrophoretic profile, combination of urea/thiourea/CHAPS were shown to remain a better solubilization buffer and additive, but the differences with sodium deoxycholate buffer was not significant. Furthermore, a deeper mass spectrometric and statistical analyses on the identified proteins and peptides from all the evaluated solubilization buffers revealed that sodium deoxycholate had similar efficiency in solubilizing proteins from oil palm mesocarps. However, it was worth to note that observed limitations to the application of sodium deoxycholate in protein solubilization were the interference with protein quantitation and only 70% of the total identified proteins were shared between the buffers. The former limitation could be rectified through a 4-fold dilution but further works are needed to determine the importance of the remaining 30% unique proteins. All the proteomics data are available via ProteomeXchange with identifier PXD013255. In conclusion, sodium deoxycholate is applicable in the solubilization of proteins extracted from oil palm mesocarps to an efficiency level comparable to that of urea/thiourea/CHAPS buffer.

EXTERNAL LINK

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

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Protein extraction from mesocarp

- 1 10 g of sliced mesocarps were ground and mixed well with 25 mL of cold acetone containing 10% trichloroacetic acid and 1 mM dithiothreitol on ice.
- 2 The slurry was then centrifuged at 13,000 g for 10 min at 4°C (RA-300 rotor, Kubota 7820, Kubota Corporation, Tokyo, Japan).
- 3 The washing step was repeated once before adding 25 mL of cold 80% methanol containing 0.1 M ammonium acetate to the precipitate; mixed well and centrifuged as before, on ice.

4 The precipitated mesocarp pellet was washed with 25 mL of cold 80% acetone.

5 The mixture was mixed well and centrifuged again at 13,000 g for 10 min at 4°C.

10m

6 Pellet was gently re-suspended in 15 mL of extraction buffer containing 0.7 M sucrose, 1 M Tris-HCl, pH 8.3, 5 M NaCl, 50 mM DTT, 1 mM EDTA and a tablet of Roche protease inhibitors.

Protein extraction from mesocarp

30m

7 The resuspension was sonicated using ultrasonic bath for 30 mins (Townson & Mercer Ltd., England, UK).

8 The mixture was then sieved through two layers of Miracloth (Calbiochem, EMB Millipore Corporation, Billerica, MA) to separate non-macerated plant materials.

15m

9 An equal volume of fresh 50 mM, pH 8.0 Tris-saturated phenol (15 mL) was added to the mixture, mixed well and centrifuged at 15,000 g for 15 min at 4°C (RA-300 rotor, Kubota 7820) for phase separation.

10 Proteins in the upper phase were precipitated by adding five volumes of cold ammonium acetate-saturated methanol (25 mL) to one volume of pheno phase, mixed well and incubated at -20°C overnight before being centrifuged at 15,000 g for 15 min at 4°C (RA-300 rotor, Kubota 7820).

11 The protein pellets were then rinsed with 5 mL of cold ammonium acetate-saturated methanol and washed three times with 5 mL of cold 80% acetone. The protein pellet was air-dried for 5 min.

Solubilization of protein pellet

12 A volume of 600 µL of each evaluated buffers was added to the protein pellet. Buffer A: Urea/thiourea/CHAPS – 7 M urea, 2 M thiourea, 4% CHAPS, 0.4% DTT, 10 mM Tris; Buffer B: Urea/CHAPS – 7 M urea, 4% CHAPS, 0.4% DTT, 10 mM Tris; Buffer C: Urea/sodium deoxycholate – 7 M urea, 4% sodium deoxycholate, 0.4 % DTT, 10 mM Tris; Buffer D: Sodium deoxycholate – 4% sodium deoxycholate, 0.4% DTT, 10 mM Tris.

13 Commercially available 2D Quant Kit (GE Healthcare Life Sciences, Uppsala, Sweden) was then utilized to determine protein content in the samples. Bovine serum albumin provided with the kit was used as the protein calibration standard and each quantitation was performed in duplicate.

14 A 4-fold dilution was performed on the proteins solubilized with sodium deoxycholate-containing buffers when Pierce™ 660 nm Protein Assay Reagent (Thermo Scientific, IL, USA) or Coomassie-based Bradford was used.

In-solution protein digestion

15 To obtain 50 µg of proteins for digestion, the solubilized proteins were re-precipitated with cold ammonium acetate-saturated methanol.

16 The precipitated proteins were re-suspended in 0.1 M ammonium bicarbonate and 1 M urea before reduction and alkylation using 50 mM tris(2-carboxyethyl)phosphine and 150 mM iodoacetamide, respectively.

17 Sodium deoxycholate (1% w/v) was added to the protein solution prior to digestion with 4 µg of modified sequencing grade trypsin (Promega, Madison, WI, USA) in 50 mM NH₄HCO₃ for 16 h at 37°C.

- 18 Sodium deoxycholate was removed after tryptic digestion by acidification using 0.5% formic acid and centrifugation at 14 000 g (RA-300, Kubota 7820) for 15 min at ambient temperature.
- 19 The peptide solution was then dried in a centrifugal evaporator (CentriVap Concentrator, Labconco, MO, USA).
- 20 The dried peptide pellet was resuspended in 200 µL of 0.1% formic acid.
- 21 Acetonitrile, methanol and 0.1% formic acid-conditioned Empore solid phase extraction disks (3M Purification, Inc., MN, USA) were added to the peptide solution and incubated at ambient temperature with slight agitation for 4 h.
- 22 The bound peptides on the C18 membrane disks were sequentially eluted with 50% ACN in 0.1% FA for 2.5 h.

One-dimensional gel electrophoresis

- 23 To obtain 100 µg of proteins for electrophoretic separation, the solubilized proteins were re-precipitated with cold ammonium acetate-saturated methanol.
- 24 The precipitated proteins were then dissolved in Laemmli buffer (62.5 mM Tris-HCl, pH 6.8, 2% SDS, 25% glycerol, 0.01% bromophenol blue, 0.005% β-mercaptoethanol) and denatured by boiling at 95°C for 4 min.
- 25 100 µg protein was loaded into each lane on a 1.0 mm in-house casted 12% polyacrylamide gel.
- 26 4. Electrophoresis was conducted in a Bio-Rad mini-PROTEAN® Tetra Cell apparatus (Bio-Rad Laboratories Inc., Hercules, CA) at 200 V for 1 h.
- 27 5. Following electrophoresis, the separated proteins were fixed for 30 min in a fixing solution (50% ethanol, 10% acetic acid) and stained with an in-house prepared Colloidal Coomassie G-250. The gel was destained with Milli-Q water until the gel background was clear.
- 28 6. The gel was scanned as digital image using Bio-5000 Plus scanner (Microtek, Hsinchu, Taiwan) according to the manufacturer's instructions.

Liquid chromatography-tandem mass spectrometry

- 29 1. Tryptic digests were reconstituted in 20 µL of 0.1% formic acid and 5% of acetonitrile.
- 30 2. 2 µL of reconstituted digests was loaded onto Acclaim PepMap 100 C18 reversed phase column (3 µm, 0.075 x 150 mm) for peptide separation.
- 31 3. The column was equilibrated with 95% of 0.1% formic acid (mobile phase A) and 5% of 80% acetonitrile and 0.1% formic acid (mobile phase B). A gradient of 5-35% mobile phase B in 70 min was employed to elute the peptides at a flow rate of 300 nL/min.
- 32 4. Peptide ions were generated with a spray voltage of 1800 V and scanned from m/z 310 -1800. The resolving power used was 70,000. Maximum injection time applied was 100 ms. Only peptide precursors with charge state of 2-8 were fragmented.

- 33 5. Fragmentation (tandem MS) conditions consisted of scan rate with resolving power of 14,500 and 0.7 m/s isolation window. Maximum injection time used was 60 ms. Mass range scanned was from m/z 110 -1800. Fragmentation energy was 28% for both the CID and HCD.

Data analysis

- 34 1. Data acquisition in positive mode was executed with Xcalibur Version 4.1.31.9. Generated RAW data was processed with Proteome Discoverer 2.1.
- 35 2. Tandem mass spectra were searched with SEQUEST HT against *Elaeis guineensis* and *Phoenix dactylifera* taxonomies in NCBI protein database.
- 36 3. Mass tolerances for peptide and product ions were set to 20 ppm and 0.5 Da. Trypsin was designated as the protease with two missing cleavages allowed. Carbamidomethylation on cysteine and lysine was set as fixed modifications while oxidation of methionine and deamidation of asparagine and glutamine were searched as variable modifications. Proteins were accepted if they had at least one Rank 1 peptide.
- 37 All database searches were also performed against the decoy database to determine the false discovery rate. All peptide spectral matches were validated using the Percolator version 2.04 based on *q*-value at 1% false discovery rate.
- 38 Venn diagram of the identified proteins was created using a free web-based program (<http://bioinformatics.psb.ugent.be/webtools/Venn>). Gene ontology was annotated using the Retrieve/ID mapping tool in Uniprot.

Supervised partial least squares-discriminant analysis (PLS-DA)

- 39 1. Metaboanalyst 4.9 was employed to perform the PLS-DA. Data inputs containing measured mass, retention time and intensity were extracted from the RAW files.
- 40 2. Four replicates were used. Peaks of the same group were summed. For peak matching, variables were grouped based on their retention time. Mass and retention time were set at 0.025 m/z and 30 secs. Interquartile range was used for filtering. Normalization and data scaling were performed using the sum of intensities and Pareto scaling.
- 41 3. Statistical model was validated using permutation test.



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