APPENDIX
A - I. DNA Extraction Method [van Burik et al., 1998]

Fungal mycelium (approx. 1 g wet wt.)

↓

Ground to powder in liquid nitrogen

↓

Add 0.5mL extraction buffer (preheated to 65°C) + 100 μL 1% β-mercaptoethanol

↓

Mix vigorously

↓

Incubate at 65°C for 30 min

↓

Add 100 μL of 3M sodium acetate

↓

Centrifuge at 10,000 rpm for 10 min

↓

Supernatant + equal volume of phenol (pH 8): CHCl₃: isoamyl alcohol (25:24:1)

↓

Mix vigorously

↓

Centrifuge at 10,000 rpm for 15 min

↓

Supernatant + equal volume of CHCl₃: isoamyl alcohol (24:1)

↓

Mix

↓

Centrifuge for 10min

↓

Supernatant + 200 μL of RNase (1 mg mL⁻¹ stock)

↓

Incubate at 37°C for 30 min

↓

A-2
Equal volume of phenol (pH 8) : CHCl₃ : isoamyl alcohol (25:24:1)

Mix vigorously

Centrifuge at 10,000 rpm for 15 min

Supernatant + equal volume of CHCl₃ : isoamyl alcohol (24:1)

Mix

Centrifuge for 10 min

Supernatant + 2.5 volumes of chilled isopropanol

Centrifuge at 10,000 rpm for 10 min at 4°C

Wash with 70% cold ethanol

Dissolve in minimum volume of sterile d/w

Store in −20°C

**Extraction buffer:** Tris HCl 100 mM

Na₂EDTA/Na₄EDTA 20 mM

CTAB 2 % (w/v)

NaCl 1.4 M

Poly vinyl pyrolidine 1 % (w/v)

**T.E buffer**

Tris-HCl (pH 8) 10 mM

EDTA 1 mM
A - II. DNA Standard

DNA (HiMedia, Cat. RM 511) was dissolved in sterile distilled water to get the desired concentration and the absorbance was read at 260 nm.

\[
y = 0.0076x + 0.1259 \\
R^2 = 0.9972
\]
A – III. Protocol for the intracellular protein extraction using Sigma total protein extraction kit [Cat. PROT-TOT]

Fungal biomass

Grinding in liquid nitrogen in a mortar and pestle to a fine powder

100 mg crushed biomass

+ 2 mL Chaotrophic Extraction Reagent 1 (Cat. C 0481)

+ 0.2 mL Protease inhibitor cocktail (Cat. P 2714)

Incubate for 30 min. at 30°C

Centrifuge at 10,000 rpm for 30 min at 15°C

Decant supernatant into a clean tube and discard the insoluble pellet

Supernatant + 50 μL of Tributylphosphine solution (200 mM) (Cat. T 7567)

Incubate at room temperature for 1 hour

Add 60 μL of Iodaacetamide (500 mM) (Cat. A 3221)

Incubate at room temperature for 1.5 hours

Centrifuge at 10,000 rpm for 5 min.

Supernatant

Protein estimation

Electrophoresis
A - IV. Urea buffer protein extraction method

[Optimization of protein extraction from Aspergillus nidulans for gel electrophoresis]

Lyophilized fungal biomass powder in 0.2 mL urea sample buffer

1. Mix vigorously
2. Boil for 2 min.
3. Vortex for 1 min.
4. Boil for 1 min.

**Urea buffer** — 1 % SDS
9 M Urea
25 mM Tris-HCl pH 6.8
1 mM EDTA
0.7 M mercaptoethanol
A – V. **Protein standard** [Lowry *et al.*, 1951]

Bovine Albumin (BSA) (SRL chemicals, Cat. 0140299) dissolved in d/w was used as standard for protein. The dilutions required were done using d/w and the absorbance was read at 660 nm on a Shimadzu spectrophotometer (Model UV 1201V)
A - VI. SDS PAGE ---- Reagents and Gel Compositions [Laemmli, 1970]

Acrylamide / Bis: Acrylamide - 29.2%  
N'N'-bis-methylene-acrylamide - 0.8% in d/w

10% (w/v) SDS in d/w

Resolving buffer: 1.5 M Tris-HCl pH 8.8 (adjust pH with 6 N HCl)

Stacking buffer: 0.5 M Tris-HCl pH 6.8 (adjust pH with 6 N HCl)

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<thead>
<tr>
<th>% gel</th>
<th>D/w mL</th>
<th>Acrylamide/bis mL</th>
<th>Gel buffer mL</th>
<th>10% SDS mL</th>
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<td>5.7</td>
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10% Ammonium persulphate: freshly prepared

For 10 mL gel volume:

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<tr>
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Sample buffer (SDS reducing buffer) 10X electrode running buffer, pH 8.3

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<tr>
<th>Component</th>
<th>Concentration</th>
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<tr>
<td>D/w</td>
<td>3.55 mL</td>
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<tr>
<td>0.5M Tris-HCl, pH 6.8</td>
<td>1.25 mL</td>
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<tr>
<td>Glycerol</td>
<td>2.5 mL</td>
</tr>
<tr>
<td>10% SDS</td>
<td>2.0 mL</td>
</tr>
<tr>
<td>0.5% (w/v) bromophenol blue</td>
<td>0.2 mL</td>
</tr>
<tr>
<td>Total volume</td>
<td>9.5 mL</td>
</tr>
<tr>
<td>Tris base</td>
<td>30.3 g/L</td>
</tr>
<tr>
<td>Glycine</td>
<td>144 g/L</td>
</tr>
<tr>
<td>SDS</td>
<td>10 g/L</td>
</tr>
</tbody>
</table>

Use 1X buffer for the electrophoresis run.
**Silver staining protocol** [Heukeshoven & Dernick, 1985]

Electrophoresed gel

1. Fix* the gel in Fixative solution for at least 1 h
2. Wash* gel with methanol (20 min x 2-3 times)
3. Treat* with 0.02 % Na$_2$S$_2$O$_3$ for 1 min.
4. Rinse the gel with d/w
5. 0.2 % Silver Stain solution* for 30 min. (place in dark)
6. Rinse the gel with d/w to wash off excess silver stain solution
7. Add developer solution till proteins bands appear
8. Fixative for 10 min
9. Wash the gel with d/w

* The gel was placed on gel rocker during these steps.
**A – VIII. Reagents for Western blotting**

**Transfer buffer:**
- Tris - 3.03 g
- Glycine - 14.4 g
- Dist. Water - 800 mL
- Methanol - 200 mL

**TBST buffer:**
- Tris - 2.42 g
- NaCl - 8 g
- Tween 20 - 500 μL
- Dist. Water - 200 mL

Adjust pH to 7.2 to 7.5 and make up volume to 1000 mL using dist. Water

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**A – IX. Czapek Dox (CD) medium composition (with modification)**

- Glucose* - 10 g
- Sodium nitrate - 3 g
- Dipotassium hydrogen phosphate - 1 g
- Magnesium sulphate - 0.5 g
- Potassium chloride - 0.5 g
- Ferric sulphate - 0.02 g
- Sea water - 1000 mL

Wherever required, milk solution (prepared using skimmed milk prepared in d/w) was added to this basal medium, after autoclaving separately.

* in original composition, sucrose is used instead of glucose.
A - X. Staining reagents used for visualization of humic substances, proteins and exopolysaccharides (TEP) in sediment extracts

   Equal volumes of 10 % aq. sodium nitrite and 5 g of benzidine in 25 mL conc. HCl diluted to 1 : 1

   Stock solution - 1 % (w/v) in sterile d/w
   Working solution - Dilute the stock 25-fold in 0.2 μm filtered seawater to 0.04% concentration and pH 7.4

3. Alcian blue stain for TEP [Long & Azam, 1996]
   0.02 % alcian blue in 0.06 % acetic acid (pH 3.3)
4 - Methylumbelliferone (Sigma Chemicals, USA, Cat. M 1381) was dissolved in methycellosolve to get the desired concentrations. The fluorescence was measured at 340 nm excitation and 450 nm emission on a Shimadzu spectrofluorimeter (Model RF1501).

The standards were done for 1-10 mM and 10 – 100 mM MUF concentration range.