Supporting Information

Structural and Nanomechanical Properties of *Termitomyces clypeatus* Cell Wall and its Interaction with Chromium (VI)

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Preparation of mycelia. *T. clypeatus was* grown in 75 mL complex medium taken in 250-mL Erlenmeyer flask under static condition at 30 ºC for 7 days. The growth medium contained 10% (v/v) potato extract and the following ingredients [(w/v)]: 1% malt extract, 0.057% boric acid, 0.15% KH_2PO_4, 0.037% $CaCl_2.2H_2O$, 0.05% $MgSO_4.7H_2O$, 0.0036% $MnCl_2.4H_2O$, 0.03% $ZnSO_4.7H_2O$, 0.025% $FeSO_4.7H_2O$ and 0.004% $CuSO_4.5H_2O$. At the end of incubation, mycelia were harvested from the fermented broth by filtration and washed with deionised water.

Transmission electron microscopy (TEM). *T. clypeatus* mycelia before and after adsorption process were thoroughly washed with deionized water, and then fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 4 h. These were then fixed in 1% OsO_4 in phosphate buffer at 4 °C for 60 min and finally dehydrated with ethanol and embedded in spurr resin.

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The samples were thin sectioned (50 nm) in ultramicrotome using a diamond knife, and the sections were collected on copper grids. However, instead of staining ultrathin sections with uranyl acetate and lead citrate, electron scattering provided by the adsorbed metal ions acted as the contrasting agent. Electron micrographs were recorded on HRTEM (JEOL JEM 2010) instrument, equipped with energy dispersive X-ray analysis (EDXA). Micrographs were analyzed from multiple samples.

Atomic Force Microscopy. Atomic force microscopic (AFM) images of *T. clypeatus* mycelia were recorded in tapping mode at ambient condition using a multimode AFM (Veeco Metrology, Autoprobe CP-II, Model No AP0100) with silicon probes (RTESPA-M, Veeco, Santa Barbara, CA). The cantilever with long tips (aspect ratio 4:1) with spring constant 20 N/m and resonance frequencies of 245-285 kHz, was used to image the surface morphology of the mycelia. The force applied by the scanning tip was controlled to avoid dissection of the samples which were scanned in both front and back directions several times before recording an image to ensure minimal effects from non-linearity, such as hysteresis. AFM imaging was recoded initially by scanning a 20 µm × 20 µm area that contains several fungal mycelia. The image size was gradually reduced to isolate a single mycelium and finally obtain high resolution images (1 µm × 1 µm) to isolate individual cells. The images were recorded from the randomly selected position on a single mycelium. Mycelium was scanned in both forward and backward directions before imaging to ensure tip artifacts, such as hysteresis, were not altering the images. The reproducibility of the images was verified by recording the images on different hyphae grown in separate experiments. On completing image recording, an offline section analysis was performed for each image to obtain informations on the sample

height and surface roughness. The surface roughness of the sample was measured using ProScan Image Processing Program provided by the manufacturer. The root-mean-square average of the surface roughness (R_{rms}) was measured with the following expression

$$R_{rms}=\sqrt{\frac{\sum(Z_i - Z_{avg})^2}{N_p}}$$

where Z_i is the current Z value, Z_{avg} is the average of the Z values within the given area, and N_p is the number of point within the given area.

The phase images providing complementary methodology to topographic imaging yielded information on sample heterogeneity and were taken simultaneously with the height or the topographic images.

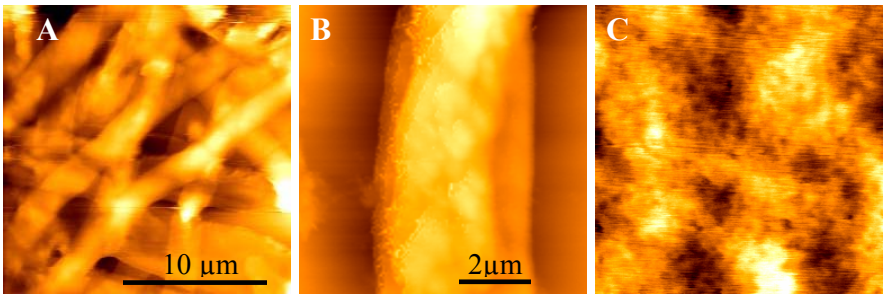

Figure S1. Atomic force microscopic images (A) containing several of *T. clypeatus* mycelia; (B) a single mycelium; and (C) high resolution image depicts surface ultrastructure.

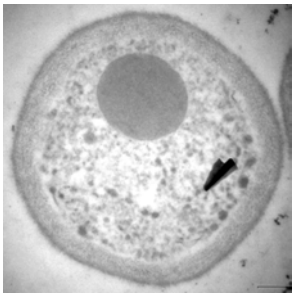

Figure S2. TEM micrographs of *T. clypeatus* mycelia harvested from stationary growth phase.

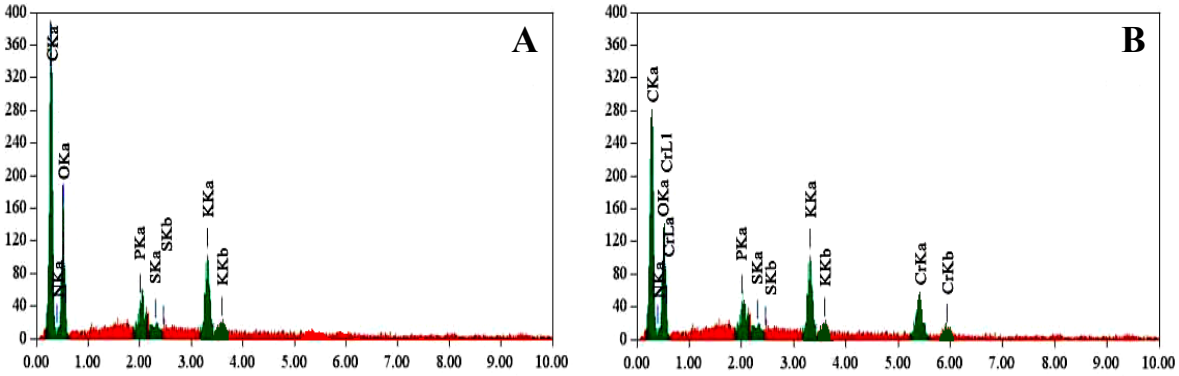

Figure S3. EDXA profile of control (A) and chromium (B) adsorbed mycelia.

Energy dispersive X-ray analysis (EDXA) shows the presence of C, N, O, Na, K, P and S (Figure S3A) in the control mycelia. These signals are due to X-ray emissions from the polysaccharides and proteins present on the cell wall of the mycelia. Additional signals of Cr are noted in the post adsorbed mycelia (Figure S3B) indicating the binding of metal ions on the mycelial surface.

Table S1. Force distance components of nonlinear region of the extension portion of the force cycle recorded on *T. clypeatus* mycelia harvested from different growth phases

Growth phase	Distance (nm)	Force (nN)
Early logarithmic phase	150 ± 25.5	3
Mid logarithmic phase	100 ± 10.5	1.8
Stationary phase	30 ± 8.4	0.1
Death phase	50 ± 9.2	0.25

Data represent an average of five independent experiments ± SD shown by error bar.

Table S2. Values of radius (R) and cell wall thickness (h) of *T. clypeatus* mycelia harvested from different growth phases and after adsorption process

Growth phase	Before Adsorption		After Adsorption	
	R (μm)	h (μm)	R (μm)	h (μm)
Early Log	1.34 ± 0.12	0.187 ± 0.02	1.37 ± 0.13	0.187 ± 0.03
Mid Log	1.75 ± 0.15	0.196 ± 0.025	1.9 ± 0.15	0.191 ±0.04
Stationary	1.98 ± 0.18	0.208 ± 0.04	2.35 ± 0.19	0.195 ± 0.01
Dead	1.78 ± 0.12	0.2 ± 0.03	2.1 ± 0.15	0.199 ± 0.02

Data represent an average of five independent experiments ± SD shown by error bar.