Electronic Supplementary Material (ESI) for RSC Advances.

Fungal chitosan-copper as a rational and sustainable nanozyme with intrinsic laccase activity for robust degradation of phenolic pollutants

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Electronic Supplementary Material

Chitosan extraction procedure

The method used for chitin and chitosan extraction in this work followed the procedures described in elsewhere.[1] Briefly, the mycelia recovered by filtration through Whatman filter paper was subjected to repeated washing with distilled water and dried at 65 °C to a constant weight. Finely powdered dry fungal mycelia was then suspended in a 1 mol/L sodium hydroxide (1: 30 w/v), autoclaved for 20 minutes at 121 °C, and then centrifuged (6000 g ,15 min.) to separate the alkali-insoluble fraction (AIF). The AIF was then washed with distilled water, and centrifuged to a neutral pH. The residues were further extracted using 2 % acetic acid (1: 40 w/v) at 95 °C for 8 h. The extracted slurry was centrifuged at (6000 g ,15 min.) and the insoluble acid fraction was discarded. The solution was centrifuged at (12000 g ,15 min.) and the pH of the supernatant was corrected to 10 with 2 mol/L sodium hydroxide. The precipitated chitosan was then washed with distilled water, 95% ethanol, and acetone, respectively, and dried at 60 °C to a constant weight.

The resulting chitosan was checked for its degree of deactylation and presence of important functional groups using Infrared absorption technique (FTIR Nicolet XX). First a 2 mg sample of chitosan was subjected to a KBr pellet (100 mg KBr) followed by scanning the absorbance from 400- 4000 cm^{-1} Then the ratio of the absorbance at 1655 and 3450 cm^{-1} (A1655/A3450.) after appropriate baseline subtraction was taken for calculation of the degree of deactylation (DDA) according to equation 1

$$DA = \frac{A_{1655}}{A_{3450}} \times 115 \qquad \textbf{Equation (S1)}$$

In these equations 1655 and 3450 cm^{-1} represent the absorbance of chitosan at 1655, 3450,cm^{-1}

Further the average molecular weight of the obtained chitosan was estimated from its intrinsic viscosity using rotating viscometer (NDJ -8s, Shanghai) The intrinsic viscosity was determined from the y-intercept of the plot of specific viscosity vs concentration of chitosan solution in 0.2 M Sodium acetate and 0.3 M acetic acid. The molecular weight of the chitosan was then estimated according to the Mark–Houwink–Sakurada equation

$$[\eta] = KM^{a} \qquad \textbf{Equation (S2)}$$

Where, M is viscosity molecular weight, K = 0.076 mL/g and a = 0.76 in this chitosan-solvent system

Characterization of fungal chitosan

Fungal chitosan produced by the potent isolate was further characterized using FTIR and SEM. The chitosan that was dried in a powder form was investigated using Fourier transform infrared (FT-IR) spectroscopy at a 4 cm−1 resolution at wave numbers ranging from 4000 to 400 cm−1. To analyze the surface morphology, scanning electron microscope were used. The sample was processed by self-coting with gold.

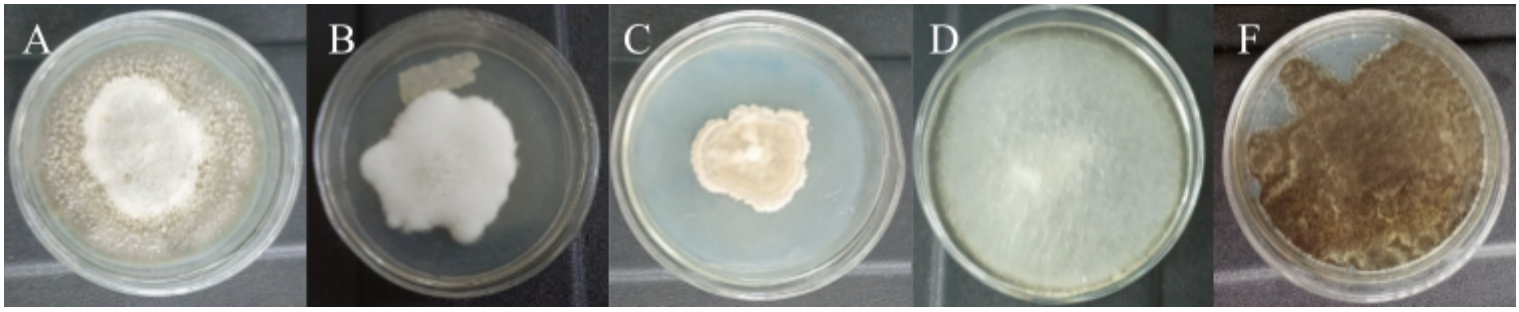

Figure S1 A representative fungal isolates cultured on PDA plates. (A) AWK1; (B) AWK2 ;(C) AWK7; (D) AWK3 and (F) AWK6

Table S1 chitosan yield by the isolated fungi in potato dextrose broth and minimal salt medium

isolate Code	**Potato dextrose broth**		**Minimal salt medium**	
	Fungal biomass (g/100mL)	Chitosan yield (mg/ g/100mL)	Fungal biomass (g/100mL)	Chitosan yield (mg/ g/100mL)
AWK1	0.4	0.0018	0.734	0.0065
AWK2	0.46	0.0031	0.561	0.0121
AWK3	0.41	0.0058	0.654	0.0124
AWK6	0.354	0.0034	0.449	0.0078
AWK7	0.37	0.0038	0.654	0.127
AWK8	0.37	0.0008		
AWK9	0.369	0.0004		
AWK12	0.467	0.0014		
AWK15	0.343	0.004		

Table S2: Taxonomic affiliation of the selected chitosan-producing fungi based on ITS rDNA sequences analysis.

Isolate Code	Top-hit Taxon	Identity (%)	Taxonomy
AWK2	*Irpex* sp. isolate FS16	>99	Basidiomycota
AWK1	*Schizophyllum commune* strain C77P	>99	Basidiomycota
AWK7	*Diaporthe* sp. isolate F255	>97	Ascomycota

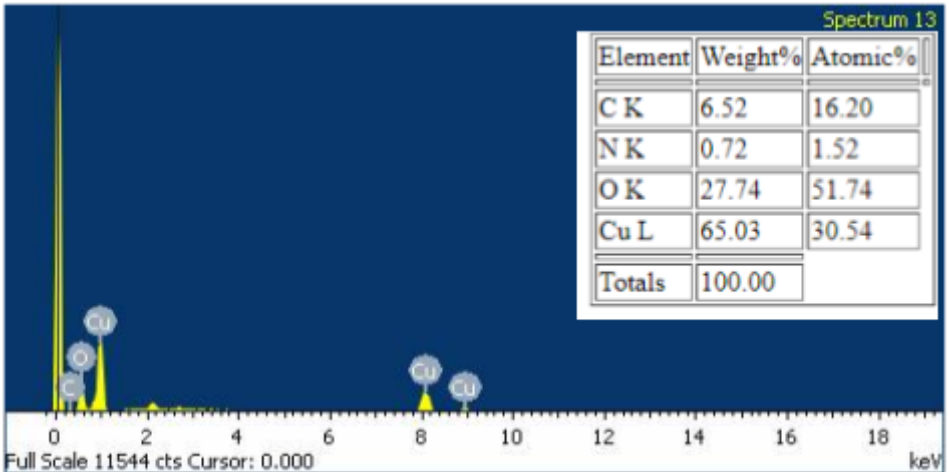

Figure S2: EDX spectrum of CsCu nanozymes

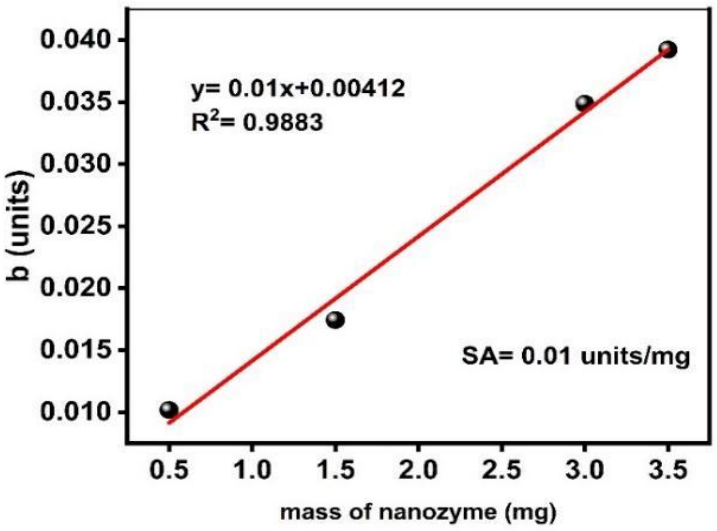

Figure S3: Activity of the CsCu nanozyme

[1] P. Pochanavanich, W. Suntornsuk, Fungal chitosan production and its characterization, Letters in Applied Microbiology 35(1) (2002) 17-21.