

## Supplementary Materials

# Evaluation of Antibiotic Biodegradation by a Versatile and Highly Active Recombinant Laccase from the Thermoalkaliphilic Bacterium *Bacillus* sp. FNT

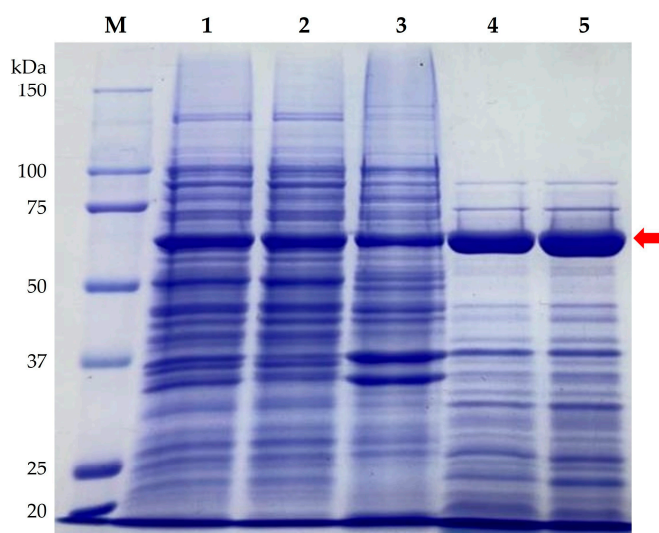
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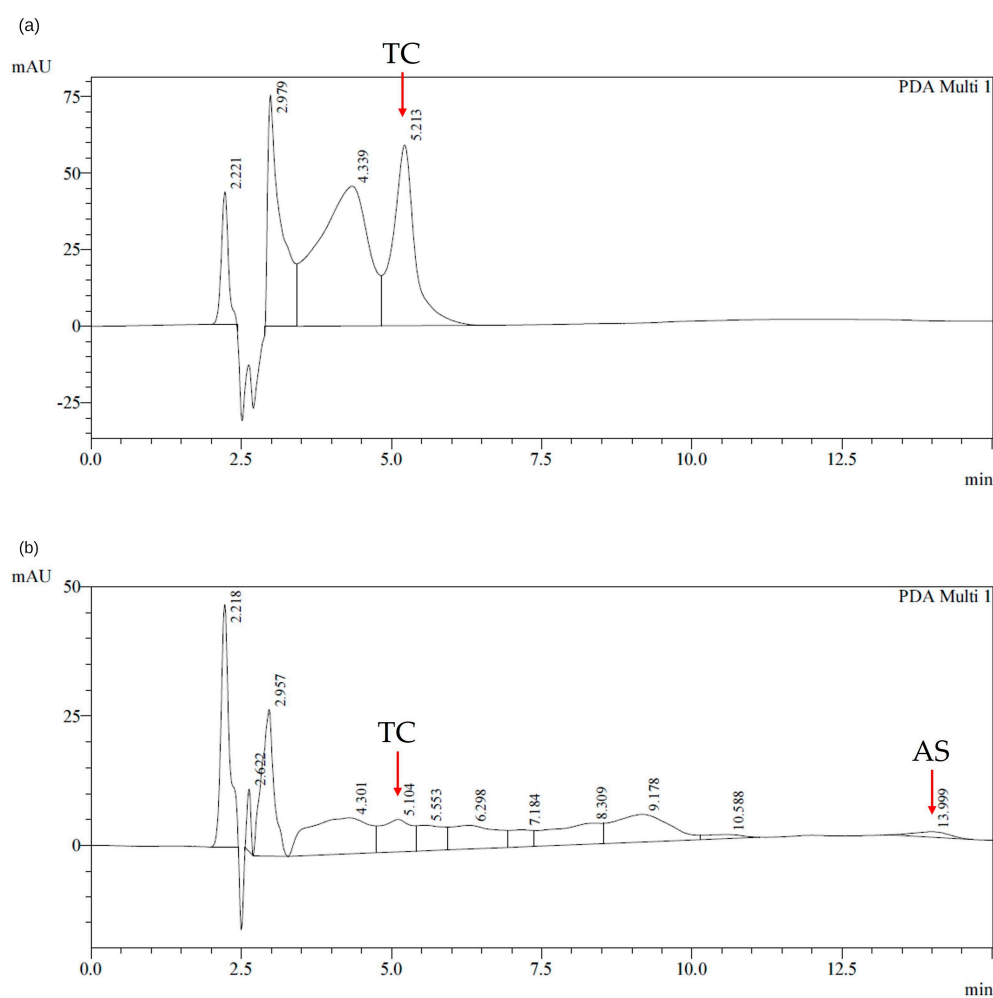
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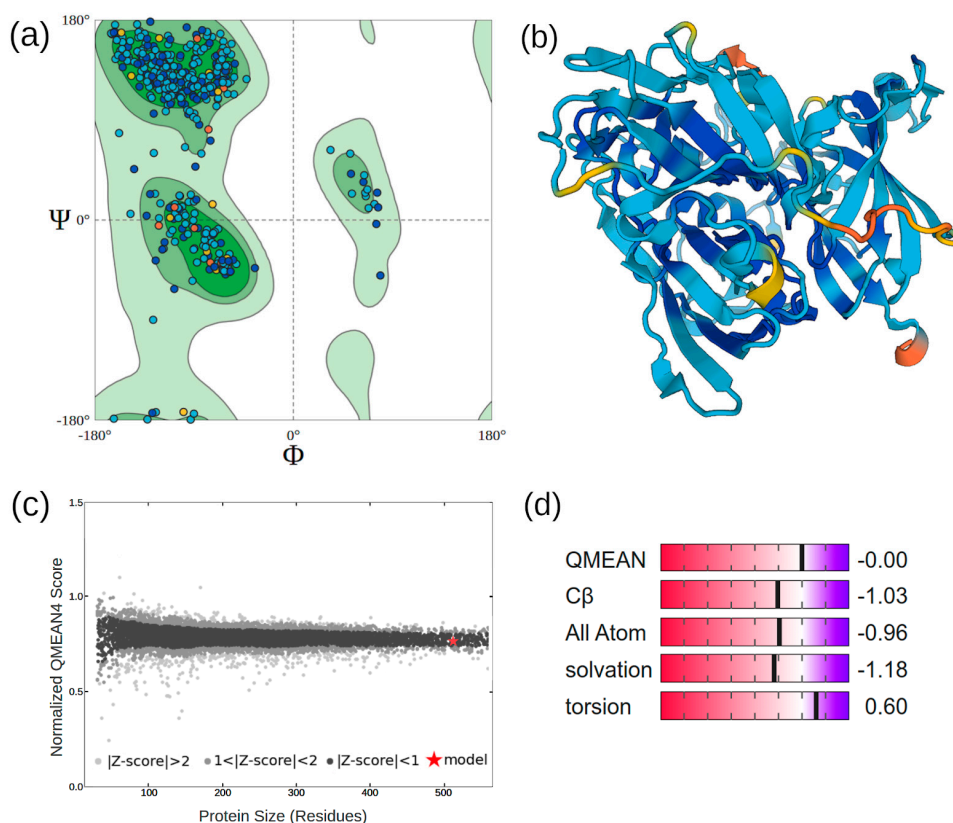
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**Figure S1.** Electrophoretic analysis of the heterologous overexpression and partial purification of FNTL. Lane M: Molecular weight marker (Biorad Precision Plus Protein™ Kaleidoscope™ pre-stained protein standard). Lane 1: *E. coli* BL21 lysate (total fraction); Lane 2: inclusion bodies solubilized with 8 M urea (insoluble fraction); Lane 3: cell-free extract (soluble fraction); Lane 4: partial purification by heat treatment at 85°C for 5 min. Lane 5: concentrated partially purified FNTL. The protein concentration loaded in each well is 15 µg and the band corresponding to FNTL is highlighted by a red arrow



**Figure S2.** Chromatograms of tetracycline separation by reversed phase HPLC. **(a)** Chromatogram obtained from control tetracycline incubated at pH 6.0, 20°C for 24 h. **(b)** Chromatogram obtained from tetracycline incubated with FNTL and AS at pH 6.0, 20°C for 24 h.



**Figure S3.** Quality assessment of the 3D model of FNTL inferred by homology with SWISS-MODEL. **(a)** Ramachandran plot of the conformational space of the three-dimensional model; **(b)** illustrating the distribution of phi ( $\varphi$ ) and psi ( $\psi$ ) angles for amino acid residues in energetically allowed and forbidden regions, and **(c)** comparison with non-redundant sets of PDB structures based on QMEAN4 scores. **(d)** Geometric evaluators of the model (C $\beta$  interaction, all-atom interaction, solvation, torsion). Ramachandran plots were generated using RamachanDraw; Z-score-transformed QMEAN4 score plots were generated using the Swiss-Model server.

**Table S1. Quality scores of FNTL three-dimensional models generated with SWISS-MODEL.** ERRAT score: percentage of the protein residues whose error value falls below the 95% rejection cutoff; Verify 3D score: percentage of residues with averaged 3D-1D score  $\geq 0.1$ ; Prosa Z-score and QMEANDisCo scores represent overall model quality. MolProbity-score represents the crystallographic resolution at which the observed quality values would be expected. Model 1 and Model 2 correspond to the models built using as templates A0A6I7FGV2 and 1GSK, respectively.

Validation tool		Swiss-Model's FNTL models	
	Scoring range	Model 1	Model 2
ERRAT (%)	[0–100]	89.33	87.29
Verify 3D (%)	[0–100]	86.35	85.85
Prosa	[–]	-7.88	-7.98
MolProbity (Å)	[0–99]	1.03	1.40
QMEANDisCo	[0–1]	0.85	0.87