Supporting Information

Characterization of an alkali- and halide-resistant laccase expressed in E. coli: CotA from Bacillus clausii

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Figure S1. Sequence alignment of cotA from *B. clausii* vs. close orthologs. Sequence alignment was performed using the guidance server (MAFFT algorithm). Alignment has been visualized in clustalX.
Figure S2. SDS PAGE gel of purified *B. clausii* cotA. Lane 1 is the purified and upconcentrated protein. Lane 2 is a 20-times dilution of Lane 1. Lane 3 is a Precision Plus Pre-stained protein standard (BioRAD), and the dotted line represents lanes that has been edited out.
Figure S3. Activities vs. pH of *B. subtilis* (red), *B. clausii* (blue) cotAs on eight substrates, including auto-oxidation profiles (green). Data is presented without subtraction of auto-oxidation, and without correction for buffer differences.
Promazine

Auto oxidation

B. clausii

B. subtilis

Caffeic acid

Auto oxidation

B. subtilis

B. clausii

Rate (485nm)

Rate (520nm)
Tannic acid

Initial Rate (355nm) vs pH

- B. subtilis
- B. clausii
- Auto oxidation

Bilirubin

Rate (440nm) vs pH

- B. subtilis
- B. clausii
**Figure S4.** Residual activity of *B. subtilis* (dotted lines, squares) and *B. clausii* (solid lines, circles) cotA measured after 5 hours incubation at pH 3-10, 50°C.
Figure S5: Residual activity of *B. subtilis* (dotted lines) and *B. clausii* (solid lines) cotA measured after 20 hours incubation at pH 5-10, 30°C. Data are in triplicate with the standard deviation error bars.
Figure S6: Michaelis-Menten analysis of *B. subtilis* cotA oxidation of DMP. Broken lines are fitted with non-competitive substrate inhibition, full lines without, giving similar $k_{cat}/K_M$. 
Figure S7: Michaelis-Menten analysis of *B. clausii* cotA oxidation of DMP. Broken lines are fitted with non-competitive substrate inhibition, full lines without, giving similar $k_{cat}/K_m$. 
Figure S8: UV-VIS absorption profile of *B. clausii* cot A. A) Full spectrum with ABS$_{280}$=1.37 B) Zoom in on the 600nm feature with ABS$_{280}$=0.052