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Environmentally friendly detoxification of rifampicin by bacterial CotA-laccase and hydrogen peroxide

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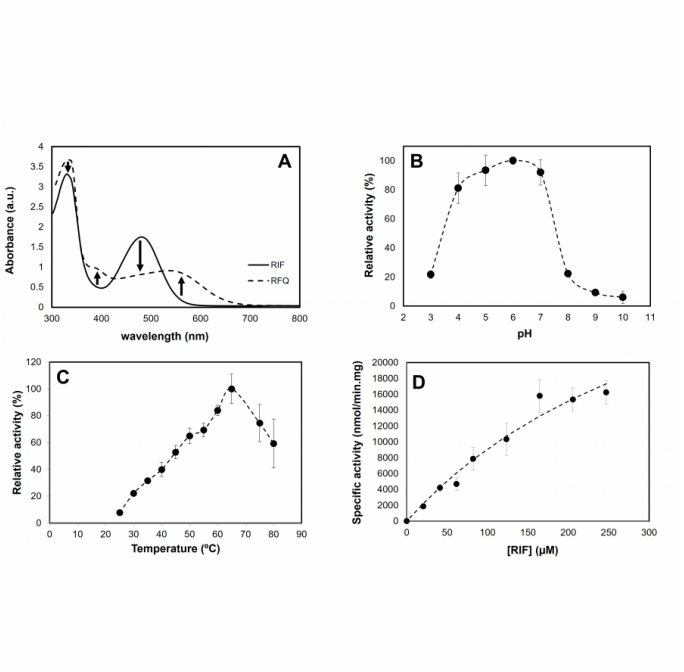
PURPOSE OF THE ABSTRACT

Antibiotics are a novel pollutant accumulating in our rivers and wastewaters ultimately leading to bacterial antibiotic resistance, a worldwide problem to which there is no current solution. We have developed a novel and environmentally friendly two-step process to transform the antibiotic rifampicin (RIF), a first-line antibiotic against tuberculosis, into a mix of non-antimicrobial compounds. The process involves a full oxidation of RIF through a reaction carried over by bacterial CotA-laccase enabling a subsequent bleaching step carried over by hydrogen peroxide into a mix of colourless final products. We have refined X-ray crystal structures of CotA-laccase soaked with rifampicin showing the antibiotic bound to the enzyme contributing to the elucidation of the molecular mechanism. Growth curves of a susceptible E. coli K12 MG1655 and four RIF resistant strains in the presence of 100 µg/mL of the final degradation products and measurements of the minimum inhibitory concentration (MIC) for the same strains show that final products are no longer an anti-microbial. Competitive fitness assays between susceptible and RIF resistant bacteria show that the final products do not exert selection pressure in favour of the resistant strains. Moreover, bioassays with the model organism C. elegans suggest that final products are not toxic to eukaryotic organisms. Overall, our results show that we have developed a robust and environmentally friendly process to effectively remediate rifampicin from antibiotic contaminated environments.

FIGURES



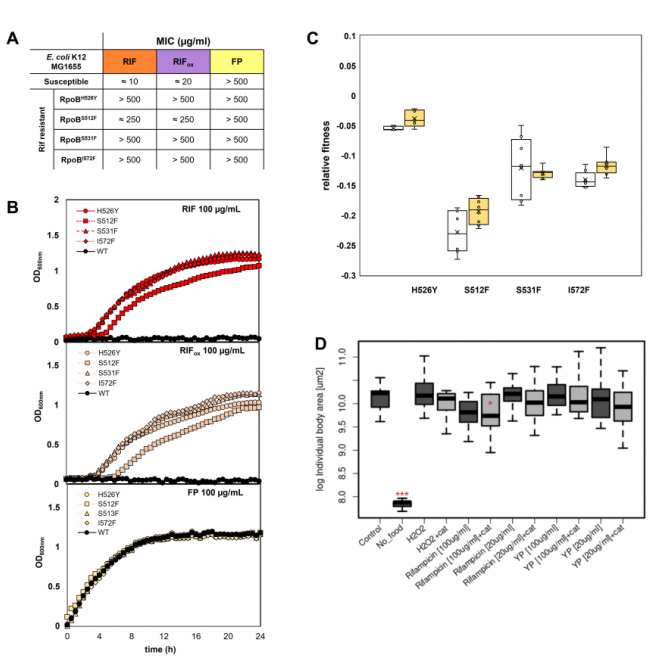


FIGURE 1

CotA oxidation of rifampicin

A) Absorbance spectra of the of rifampicin (full line) and the product of the enzymatic oxidation by CotA laccase (dashed line). B) pH profile C) Optimal temperature D) Michaelis-Menten fit of the reaction.

FIGURE 2

Assessment of the antimicrobial and toxicity properties of the degradation products

MICs (A), growth curves (B) and competitive assays (C) were performed in the presence of degradation compounds using susceptible and RIF resistant E. coli MG1655 strains. D) Toxicity of final products in a bioassay with C. elegans was also assessed.

KEYWORDS

antibiotic resistance | rifampicin | enzymatic degradation | environmentally-friendly

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