SCIENCESPRINGDAY

FACULDADE DE CIÊNCIAS E TECNOLOGIA
UNIVERSIDADE NOVA DE LISBOA

REQUIMTE/CQFB, Department of Chemistry

Biochemical and physiological insights into the bacterial cytochrome *c* peroxidase from *E. coli*

Microbial Stress and Bioremediation Group

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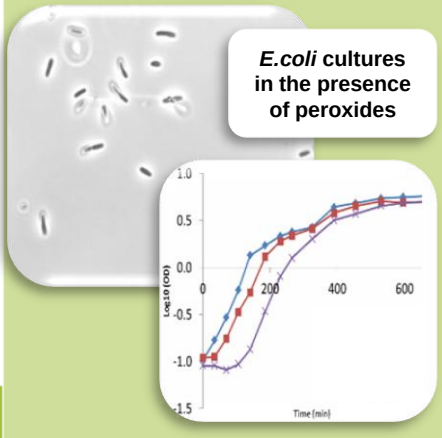
Objectives

The *yhjA* gene from *Escherichia coli* encodes a putative Cytochrome *c* Peroxidase (CCP) that has the distinct feature of containing not two but three *c*-type heme binding motifs, with the extra heme being located at an additional N-terminus domain and being proposed to be attached to the cytoplasmic membrane. A homology search shows that this gene has a high occurrence in the genome of pathogenic bacteria, but its physiological function remains unknown.

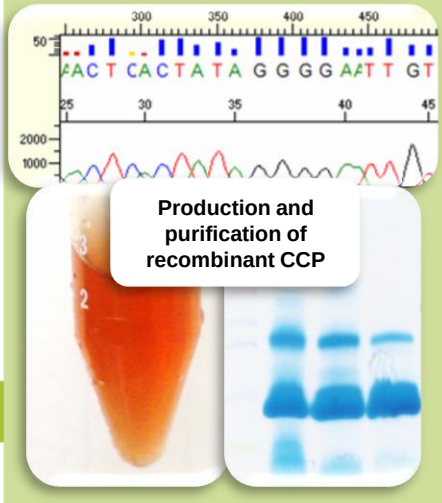
We intend to biochemically characterize the protein CCP/YhjA and its domains to unravel the function of the extra N-terminal domain and establish whether the catalytic mechanism of this enzyme is different from the one proposed for the other CCPs.



Methodology

Several aproaches will be used in order to characterize this protein:

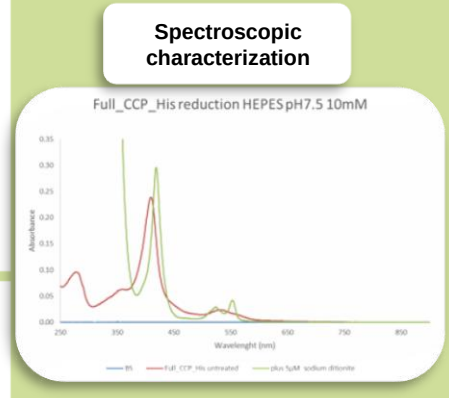
- Physiological and transcriptomic experiments to get insights into the physiological function of *yhjA* gene in response to and protection against oxidative stress;
- Production of recombinant CCP from *Escherichia coli*;
- Biophysical characterization of the *E.coli* CCP using different biochemical and spectroscopic techniques, as UV-visible, EPR and NMR spectroscopies;
- Kinetic assays to establish the peroxidase activity of *E.coli* CCP.



Expected Results

These results will allow us to propose the involvement of *E.coli* CCP/YhjA in a specific physiological pathway. By characterizing the full-length CCP and its domains we will determine whether this enzyme has a activation mechanism and if it is calcium dependent.

Overall the results will contribute to a better understanding of the enzymatic mechanism of CCP from pathogenic bacteria and their virulence (similar three-heme CCPs can be found in the genus *Salmonella* and *Yersinia*), as we believe that these enzymes constitute a promising target for drug-design, since its inhibition would make the bacteria more susceptible to the immune system response.



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