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Sustainable enzymatic approach for the release and detection of periplasmic peroxidases in *Escherichia coli*

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***Abstract**: This study explores an enzymatic approach for protein isolation, focusing on peroxidases expressed in the periplasmic space of Escherichia coli. By using hydrolytic enzymes as lysozyme for controlled cell wall degradation, we demonstrate an efficient and ecofriendly method for peroxidase release, offering advantages over traditional chemical and mechanical techniques.*

Introduction

Peroxidase is an enzyme with wide-ranging applications in biotechnology, medical diagnostics, and the food industry. Traditional production methods rely on extraction from natural sources, primarily plants or fungi. These methods involve mechanical techniques such as homogenization or sonication to release the enzyme, followed by chemical purification steps as salt precipitation, chromatography, or solvent extraction. While effective, these approaches can be costly, time-consuming, and subject to variability due to the biological nature of the source.

To overcome the limitations associated with extracting peroxidases from natural sources, we have developed an environmentally friendly method for the gradual release of recombinant expressed peroxidases in *Escherichia coli*, using lysozyme for cell disruption. In this study, peroxidase is used as a model protein to assess the effectiveness of our approach, which provides a controlled, reproducible, and scalable alternative to conventional chemical and mechanical lysis methods.

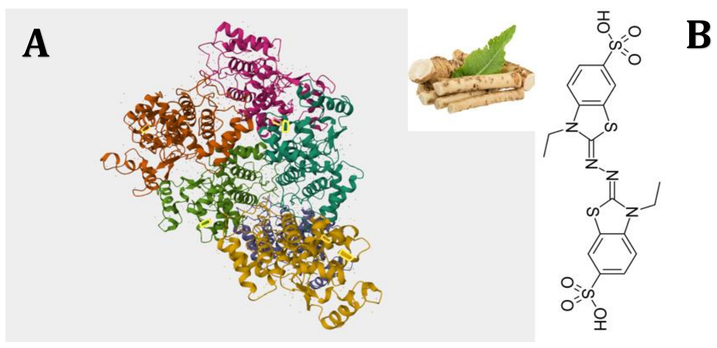

Fig.1. Protein structure of horseradish peroxidase (*Armoracia rusticana)* (A) and its substrate ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (B).

Materials and method

I. Pre-cultivation of *E.coli* cells
- Inoculation: 2 µL from stock cultures into 3mL LB+AMP (in sterile 15 mL tubes)
- Incubation: Overnight at 37°C

II. Growth of cultures and optical density adjustment
- Inoculation: 600 µL of pre-culture into 20 mL LB+AMP (in sterile 50 µL centrifuge tubes)
- Start of optical density at 600 nm : ~0.1
- Incubation: 37°C for 2h until OD 600~1.0

III. Induction of peroxidase expression
IPTG addition: add 16µL of 1M IPTG to each culture when OD 600~1.0

IV. Sampling for optimal expression time determination
- Sampling times: 0-18 h after induction
- Volume: 500 µL from each culture
- Processing: centrifuge to separate cells and supernatant
- Cells obtained after 4-5 h of expression were resuspended in 100 µL of 2 mg/mL lysozyme solution, and samples were taken at 0, 1, 2, 6, 18 hours.
- The samples were centrifuged and the presence of peroxidase in cells or in supernatant was observed using ABTS substrate.

Fig. 2. Experimental steps

Results and discussions

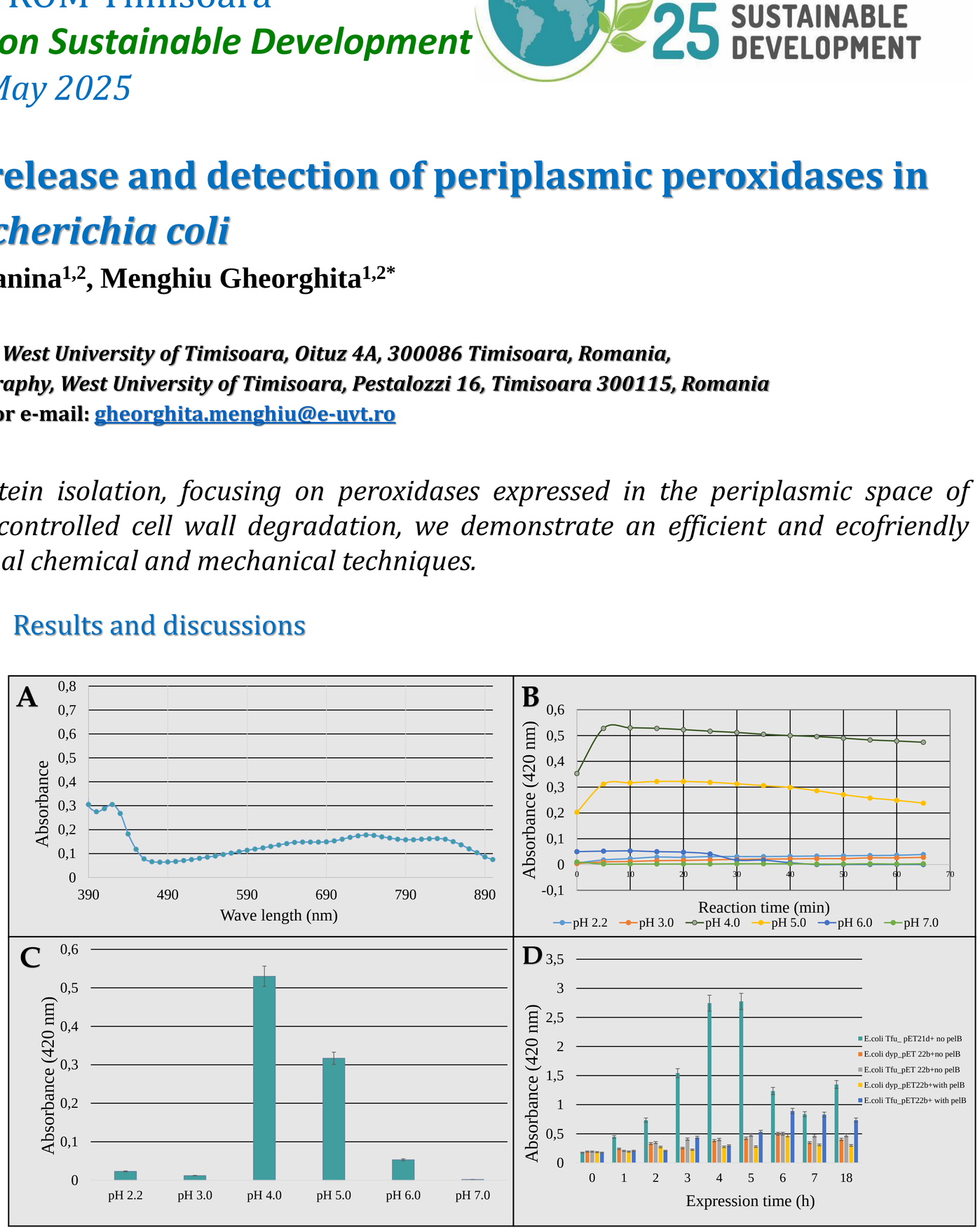

Fig.3. Absorption spectrum of $ABTS^{+}$ in the reaction with commercial horseradish peroxidase (A), Stability of the reaction product ($ABTS^{+}$) analyzed over 70 minutes (B), Optimal pH for the ABTS-peroxidase reaction (C), Optimal expression time of recombinant peroxidases in *E. coli* (D)

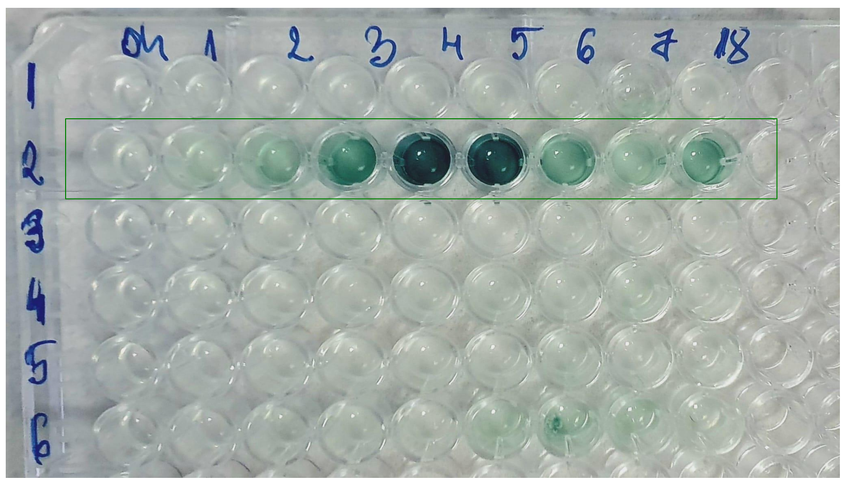

Fig.4 Recombinant peroxidase activity in 5 *E. coli* strains as a function of induction time – maximum observed in strain 2 (*E. coli Tfu*_pET21d+) after 4 h of expression

Conclusions

An optimized detection protocol was established for recombinant peroxidases expressed in *Escherichia coli,* enabling more and reproductible analysis of enzyme expression.

The optimal induction time for maximal recombinant peroxidase production was identified, contributing to improved process efficiency and expression yield.

The study demonstrated the lysozyme-mediated cell wall disruption facilitates the gradual extracellular release of recombinant peroxidases offering insight into controlled protein recovery.

The findings highlight the potential of enzymatic cell lysis as a mild, selective, and scalable alternative to conventional chemical or mechanical methods for intracellular protein isolation.

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