ID: 2400606

Viable but Non-Culturable Induction of *E. coli* by Low-Level Antimicrobials and Its Detection with AI-Enabled Hyperspectral Microscopy

Food AI + Engineering Lab
MICHIGAN STATE UNIVERSITY

MeiLi Papa[1], Aarham Wasit[2], Teresa M. Bergholz[3], and Jiyoon Yi[1]

[1]Dept of Biosystems and Agricultural Engineering, [2]Dept of Computer Science and Engineering
[3]Dept of Food Science and Human Nutrition, Michigan State University, East Lansing, MI

ASABE *Engineering a Sustainable Future*

Introduction

- Bacteria in a viable but non-culturable (VBNC) state are alive but cannot be grown on standard culture media.
- Sublethal stressors, such as antimicrobial residue, can induce VBNCs, making detection with standard methods difficult.[1]
- Hyperspectral microscopy imaging (HMI) can rapidly classify live and dead bacterial cells.[2]
- Convolutional neural network (CNN) models have been used to rapidly classify bacterial morphology.[3]

Objectives

The objectives of this study were to: **1)** induce the VBNC state in *Escherichia coli* K-12 cells under low-concentration antimicrobial stressors, **2)** obtain HMI data capturing physiological changes in VBNCs, and **3)** automate the classification of normal and VBNC cells by training a CNN model.

Materials and Methods

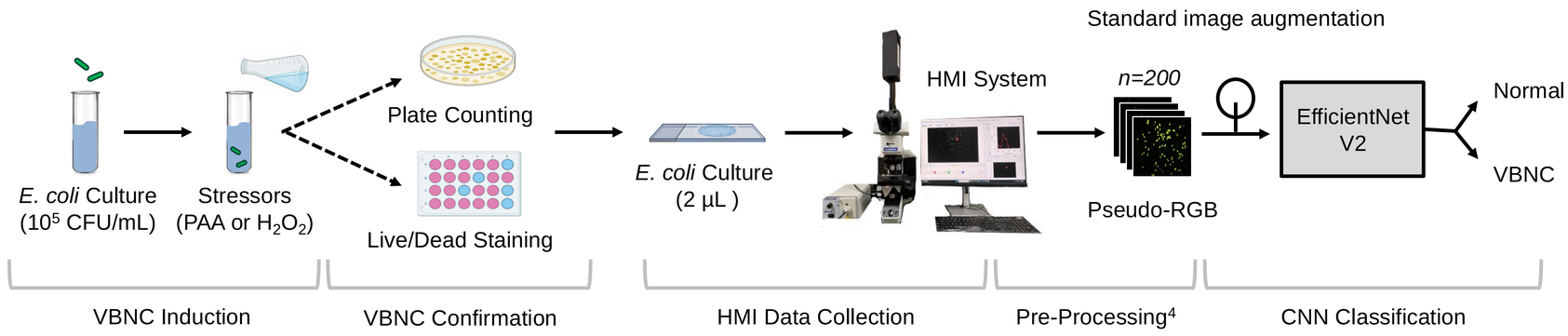


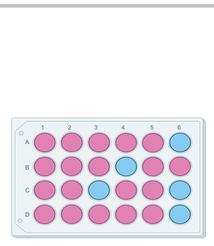
Fig. 1. Schematic diagram of overall workflow: VBNC induction and its detection by AI-enabled HMI

Stressor Concentrations
- Acidic: 0.001%, 0.01%, 0.1% peracetic acid (PAA)
- Oxidative: 0.01%, 0.1% hydrogen peroxide (H_2O_2)

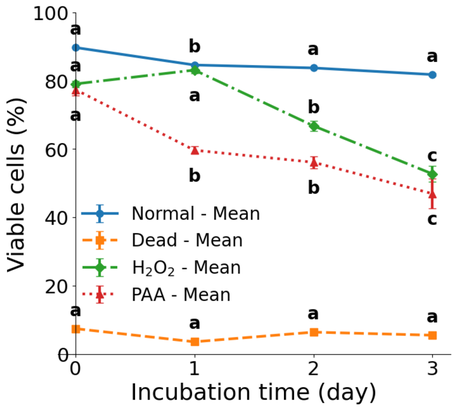
HMI System
- HinaLea 4250 hyperspectral camera + Olympus BX43
- CytoViva darkfield illuminator
- 100× oil-based objectives, exposure 200 ms, gain 16 dB

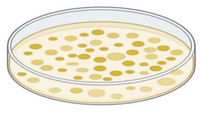
Results

VBNC Induction



Viability





Culturability

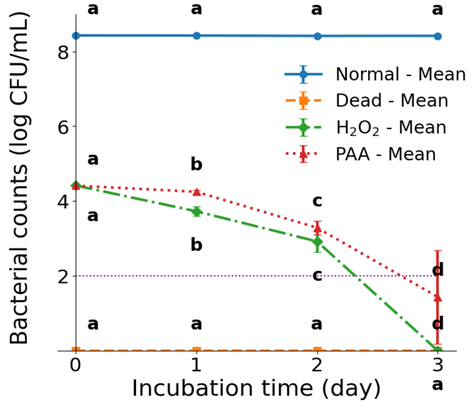


Fig. 2. Viability and culturability of stressed *E. coli* (*n=3*)

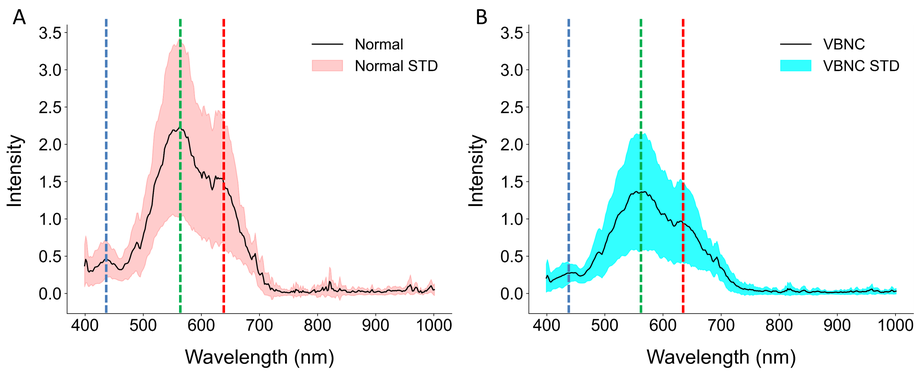


Fig. 3. Spectral profiles of normal (A) and VBNC (B) cells with 3 characteristic pseudo-RBG wavelengths

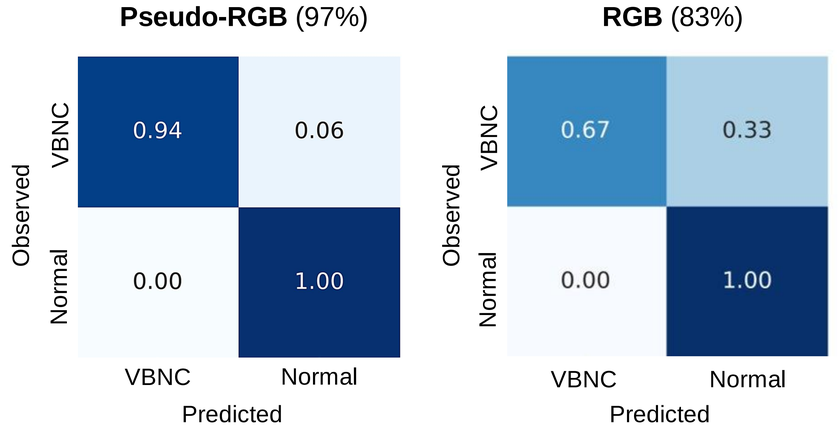


Fig. 4. Confusion matrices for model inference on testing set (*n=40*)

Conclusions

- *E. coli* VBNCs induced by exposure to low-level antimicrobials (0.001% PAA and 0.01% H_2O_2) for 3 days.
- A CNN model was trained using HMI pseudo-RGB images, achieving 97% accuracy in classifying normal and VBNC cells.
- The study highlights the potential of automating and accelerating VBNC detection by integrating HMI and CNN image classification.
- AI-enabled HMI shows strong potential for rapid pathogen detection and timely intervention to prevent foodborne illnesses and outbreaks.

Acknowledgements

This project was supported by MSU Startup Funds and MSU Research Foundation's Tetrad Award. We thank Dr. Bosoon Park (USDA-ARS) for his guidance in HMI data collection. Any opinions, findings, or conclusion expressed in this poster do not necessarily reflect the view of USDA.

References

1. Salive et al. (2020). Comparison of stress conditions to induce viable but non-cultivable state in *Salmonella*. *Brazilian Journal of Microbiology*, *51*, 1269-1277.
2. Park et al. (2023). Classification between live and dead foodborne bacteria with hyperspectral microscope imagery and machine learning. *Journal of Microbiological Methods*, *209*, 106739.
3. Yi et al. (2023). AI-enabled biosensing for rapid pathogen detection: From liquid food to agricultural water. *Water Research*, *242*, 120258.
4. Park et al. (2023). Automated segmentation of foodborne bacteria from chicken rinse with hyperspectral microscope imaging and deep learning methods. *Computers and Electronics in Agriculture*, *208*, 107802.