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"Reshaping the Future through Science and Technology"

Nucleic acid detection of live pathogens on contaminated foods

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ABSTRACT

The goal is to develop a point-of-care biosensor for the detection of live pathogens contaminating beef products. Biosensing of live pathogens is based on isothermal amplification of nucleic acid on a paper-based device. A colorimetric dye is employed as an indicator of the amplification product for visual result. The assay incorporates a compound Propidium monoazide (PMA) that makes the DNA from dead cells inaccessible for amplification. This approach is especially applicable for pathogens that can enter a viable but non-culturable state (VBNC).

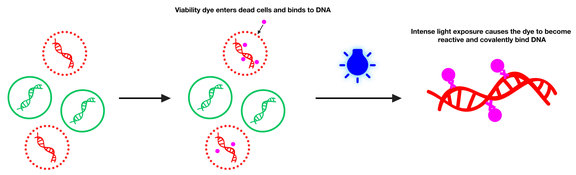

Figure 1: PMA activity (Image adapted from https://biotium.com/product/pmaxx-20-mm-in-h2o/)

BACKGROUND

PMA is a photoactive dye that can preferentially penetrate dead cells, or cells with damaged or permeabilized cell membranes, but not viable cells with intact cell membranes. Once inside the cell, PMA molecules then intercalate into the DNA and become covalently bound to DNA upon exposure to blue light. This photoactivation process results in the formation of a stable DNA-PMA complex that renders the DNA inaccessible for amplification.

MOTIVATION

The obstacle for DNA-based techniques is that DNA in the environment can remain stable and can persist for extended periods of time (days to weeks) after cell death. The biosensors based on amplification of target DNA, can detect the DNA from dead cells and lead to overestimation of contamination or false positives.

Goals

The goal is to develop a Loop Mediated Isothermal Amplification (LAMP) based colorimetric microfluidic device for the detection of *E. coli* O157:H7 DNA from live cells on contaminated food products and therefore, prevent false positives from dead pathogens.

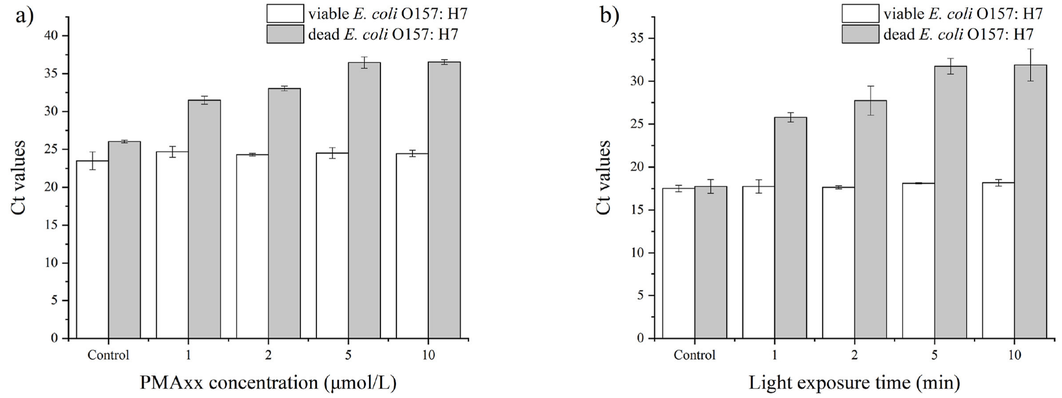

Figure 2: PMA-LAMP optimization: a) Change in Cycle threshold (Ct) value at different PMAxx concentrations. b) Change in Ct value on exposure to blue light for different durations. [1]

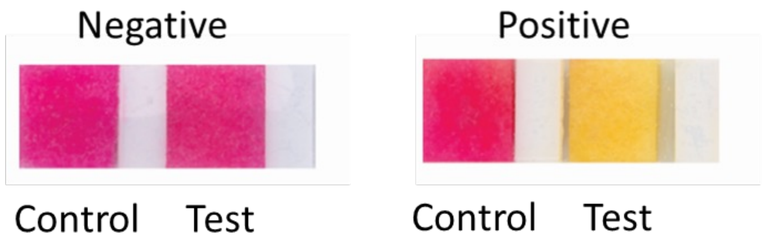

Figure 3: Paper based colorimetric RT-LAMP reaction for the detection of SARS-CoV-2 in saliva using phenol red as an indicator. Controls are RT-LAMP reactions without LAMP primers included. Test reactions have inactivated virus spiked into saliva. [2]

References

1. Lv, X. et al. Rapid and sensitive detection of VBNC Escherichia coli O157: H7 in beef by PMAxx and real-time LAMP. Food Control 115, 107292 (2020).

2. Davidson, J. L. et al. A paper-based colorimetric molecular test for SARS-CoV-2 in saliva. Biosens. Bioelectron. X 9, 100076 (2021).

PRESENTER BIO INFORMATION

Simerdeep Kaur completed her M. Sc (Hons. School) Microbiology with distinction from Panjab University in 2018. Currently, she is pursuing a PhD degree in ABE in Dr. Verma's Lab.

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