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TEMPERATURE-DEPENDENT ANTIMICROBIAL ELICITATION IN ENVIRONMENTAL BACTERIA CO-CULTURED WITH *ENTEROCOCCUS FAECALIS* IN THE PRESENCE OF AMPICILLIN/TETRACYCLINE

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Abstract

Research Objectives: *This study investigated whether co-culturing environmental bacterial isolates against Enterococcus faecalis (NTUCC 687) in the presence of ampicillin or tetracycline could elicit antimicrobial production, and whether this response was temperature-dependent.*

Methodology: *A total of 96 environmental bacterial isolates (arrayed in a 96-well plate) collected from built and natural surfaces around NTU Clifton campus were co-cultured against an Enterococcus faecalis lawn using an in-house stamping assay on UTI ChromoSelect agar. Plates were incubated at 25°C, 30°C, 37°C, and 42°C for 24–72 hours. For elicitation, UTI agar was fortified separately with ampicillin (0.05 µg/mL) or tetracycline (0.05 µg/mL). These low (sub-inhibitory) concentrations were used as*

stressors to probe signalling-associated induction of antimicrobial activity rather than direct growth inhibition. Antimicrobial activity was recorded as categorical zones of inhibition, classified as small (+), medium (++), or large (+++).

Findings: *Tetracycline elicited a higher frequency of large (+++) zones of inhibition than ampicillin, peaking at 30°C (12 isolates, 12.5%), followed by 42°C (9 isolates, 9.4%), 25°C (5 isolates, 5.2%), and 37°C (3 isolates, 3.1%). In contrast, ampicillin produced only two large zones (2.1%), both observed at 25°C. These patterns indicate that antimicrobial elicitation was modulated by both antibiotic stressor type and incubation temperature within the co-culture assay.*

Research Outcomes and Future Scope: *These findings support co-culture-based elicitation as a practical strategy for uncovering latent antimicrobial potential in environmental bacteria and highlight temperature as a key modulator of expression. Future work should identify the active producers and compounds involved, investigate underlying molecular mechanisms, and assess scalability for antimicrobial discovery.*

Keywords:

Antimicrobial Elicitation, Co-Culture, *Enterococcus faecalis*, Antibiotic Stress, Temperature