“Mechanism of antibacterial action of *trans*cinnamaldehyde against *Escherichia coli*”

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This doctoral dissertation was prepared as a series of three peer-reviewed original publications, one preprint article, and one review article. The dissertation focuses on the antimicrobial and antivirulence mechanisms of action of trans-cinnamaldehyde (*t*-CA) against pathogenic *Escherichia coli* strains. The study was conducted using strains representing two clinically significant pathotypes—uropathogenic *E. coli* (UPEC) and enterohemorrhagic *E. coli* (EHEC)—which are responsible for urinary tract infections and severe gastrointestinal diseases, respectively.

The primary objective of this dissertation was to elucidate the mechanism of action of trans-cinnamaldehyde against pathogenic *E. coli* strains. Particular emphasis was placed on evaluating the effects of *t*-CA on bacterial survival, the regulation of virulence- and biofilm-related processes, and its role in the induction of the stringent response. Additionally, the potential of *t*-CA as an adjuvant enhancing antibiotic activity was assessed, together with its efficacy in both *in vitro* and *in vivo* models.

The obtained results demonstrated that *t*-CA exhibits broad-spectrum antimicrobial activity against *E. coli* strains irrespective of their virulence profiles. Its mode of action involves disruption of bacterial cell membrane integrity, inhibition of essential biosynthetic processes, and extensive reprogramming of cellular metabolism. Exposure of bacterial cells to *t*-CA was shown to induce a RelA-dependent stringent response, the activation of which is associated with global changes in gene expression and the transition of cells into an adaptive physiological state characteristic of stress conditions.

A particularly significant finding of this work was the demonstration of the antivirulence activity of *t*-CA against EHEC strains. The compound inhibited the development of Shiga toxin-encoding bacteriophages and reduced the expression of *stx* genes, suggesting its potential to attenuate bacterial pathogenicity without increasing the severity of infection.

Furthermore, *t*-CA was found to effectively impair biofilm formation by UPEC strains, leading to a reduction in biofilm biomass and decreased synthesis of extracellular matrix components. RNA-sequencing (RNA-seq) transcriptomic analysis confirmed that the activity of *t*-CA is associated with global alterations in the expression of genes involved in energy metabolism, stress response, cellular motility, and regulatory pathways governing colonization and virulence.

An additional important aspect of this research was the evaluation of *t*-CA in combination therapy with fosfomycin. A synergistic interaction between the two compounds was demonstrated against both planktonic bacterial cells and established biofilms. The combination of *t*-CA and fosfomycin enhanced antimicrobial efficacy and limited the emergence of antibiotic resistance during prolonged bacterial exposure to fosfomycin. Moreover, experiments conducted using the *G. mellonella* infection model confirmed the protective efficacy of the combined treatment *in vivo*.

Collectively, the findings indicate that trans-cinnamaldehyde exerts pleiotropic effects on bacterial cells by combining direct antimicrobial activity with modulation of virulence-associated and stress-adaptive processes. The stringent response appears to play a central role in its mechanism of action, integrating the metabolic and regulatory changes induced within the bacterial cell. These results suggest that *t*-CA represents a promising candidate for the development of novel antivirulence strategies and combination therapies targeting pathogenic *E. coli* strains.