

Title: Antibacterial and antibiofilm properties of cinnamaldehyde against *Salmonella* Typhimurium

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Salmonella spp. leads to bacterial acute gastroenteritis, causing approximately 93.8 million human infections with 155 000 deaths annually worldwide. Biofilms are one of the sources of food contamination and its resistance to common used disinfectants is a challenge nowadays. Natural substances have been extensively studied as a strategy to microbial control. Cinnamaldehyde is well-known for its biological properties and has been widely applied as antimicrobial agent. This study evaluated the antibacterial and antibiofilm properties of cinnamaldehyde against *Salmonella* Typhimurium ATCC 14028. Broth microdilution test was performed to determine the Minimum Inhibitory Concentration (MIC) of cinnamaldehyde. *S. Typhimurium* culture was diluted to final inoculum of 5.10^5 CFU/mL and 10 μ L were transferred to 96-well microtiter plates containing cinnamaldehyde at 19 to 5000 μ g/mL. Microtiter plates were incubated at 35 °C for 24 h and MIC was defined as the lowest concentration of cinnamaldehyde required to inhibit bacterial growth. For kinetic experiments, overnight culture of *S. Typhimurium* in MHB was diluted to obtain final inoculum of 6.10^5 CFU/mL. Then, 100 μ L was transferred to tubes containing MHB supplemented with cinnamaldehyde at MIC and 2 x MIC and incubated at 35 °C for 24 h. Tubes with MHB without cinnamaldehyde were used as control. At time intervals of 0, 1, 2, 3, 4, 5, 6, 12 and 24 h, 100 μ L of each tube was removed, diluted in saline solution and plated on Mueller Hinton Agar (MHA). Plates were incubated at 35 °C for 24 h. Two-day-old *S. Typhimurium* biofilm was left to be formed on polypropylene coupons at 35 °C. Thereafter, coupons were treated with cinnamaldehyde at 2 x MIC for 1 h and cells were detached using ultra-sonic bath. Cell suspension was diluted in saline solution, plated on MHA and incubated at 35 °C for 24 h. Cinnamaldehyde inhibited bacterial growth at 312 μ g/mL. After 24 h of growth, *S. Typhimurium* population reached $\cong 9 \log_{10}$ CFU/mL. Treatment with cinnamaldehyde at MIC concentration for 24 h was decreased $\cong 2 \log_{10}$ CFU/mL and cinnamaldehyde at 2 x MIC eliminated viable cells after 12 h of exposure. After 48 h of biofilm formation, $8.03 \log$ CFU/ cm^2 was recovered from polypropylene coupons and treatment with 2 x MIC of cinnamaldehyde was able to reduce $\cong 3 \log$ CFU/ cm^2 . The results confirm cinnamaldehyde antimicrobial and antibiofilm properties, indicating this compound may be used as alternative to *S. Typhimurium* control.

Keywords: *Salmonella* Typhimurium, cinnamaldehyde, time-kill curve assay, biofilm.

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