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In Vitro Evaluation of Madio Pro+ Multipurpose Disinfectant Reveals Its Efficacy against *Salmonella typhimurium* Biofilm

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In Vitro Evaluation of Madio Pro+ Multipurpose Disinfectant Reveals Its Efficacy against Salmonella Typhimurium Biofilm

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Abstract: Biofilms are complex communities of microorganisms that adhere to surfaces and develop a protective matrix. The colonization of devices in the food and health care industries by microbial biofilms poses numerous risks. Existing antimicrobial agents often fail to penetrate the biofilm matrix, allowing the pathogen to grow and persist. The objective of this study was to evaluate the impact of MADIO PRO+ multipurpose disinfectant on Salmonella Typhimurium biofilm using crystal violet assay and FTIR spectroscopy. Results demonstrated that MADIO PRO+ multipurpose disinfectant significantly ($p<0.05$) inhibited biomass of S. Typhimurium biofilm and altered the structure of S. Typhimurium biofilm. In conclusion, MADIO PRO+ multipurpose disinfectant offers an advantage in biofilm control strategy. This discovery could help the food and health care industries to plan better microbial intervention strategies.

Keywords: salmonella typhimurium; biofilm; disinfectant

INTRODUCTION

Salmonella enterica is a Gram-negative bacterium that is rod-shaped, noncapsulated, facultatively anaerobic, and nonsporulating. It belongs to the Enterobacteriaceae family. It can be found in water, soil, and animal faeces. It is also a foodborne pathogen that has emerged as the leading cause of food-borne bacterial infection (Eng et al., 2015). Salmonellosis is a disease caused by typhoidal and nontyphoidal Salmonella serovars that were primarily responsible for food poisoning in the twentieth century. Its infection has become a major public health issue in the United States, causing an estimated 1.4 million illnesses and 600 deaths each year (Roth et al., 2018). Salmonella is the most common causative agent of gastroenteritis in Klang Valley, Malaysia, according to Nor et al. (2023). Many studies have been conducted over the last few decades to control food poisoning and other microbial infections caused by Salmonella. Biofilms of Salmonella have been shown to adhere to surfaces such as stainless steel, polyester, plastic, and aluminium (Merino et al. 2019).

Biofilms are communities of microbial cells that are adhered to a living or inert surface and encased in a self-produced extracellular polymeric matrix (Yaacob et al. 2021). Bacterial attachment initiates biofilm formation, which is followed by microcolony formation, biofilm maturation, and finally biofilm dispersion. Biofilms can be found everywhere such as restrooms, hotels, food stalls, labs, and hospitals. They aid in drug, chemical, and physical stress resistance, as well as the host immune system. Antibiotics, antifungals, and natural products are examples of potential biofilm control measures (Zawawi et al. 2020; Johari et al. 2020; Isa et al. 2022). MADIO PRO+, a multipurpose disinfectant with a novel formulation, was tested for antibiofilm efficacy against S. Typhimurium biofilm in this study.

METHODOLOGY

Microorganism

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Salmonella enterica serovar Typhimurium ATCC14028 was grown at 37 °C in nutrient broth. Culture purity was regularly confirmed by Gram staining and biochemical test. The bacterial inoculum was adjusted to an optical density (OD) of 0.7 at 600 nm before crystal violet assay.

Disinfectants

Commercial disinfectants used in this study are MADIO PRO+, chloroxylenol, sodium dodecyl benzene sulfonate, benzalkonium chloride, and sodium hypochlorite. They were tested at 25% (v/v).

Crystal violet assay

The effect of S. Typhimurium biofilm biomass following exposure to disinfectants was evaluated in a 96-wells microplate. Overnight inoculum (150 µL) and test solution (50 µL) were added to the microplate wells. An equal volume of fresh broth was added as negative control. The microplate was incubated overnight at 37 °C for 24 h. After discarding the medium, the biofilm fractions were rinsed with distilled water twice, heat-fixed at 60 °C for 30 min, stained with 0.5% (w/v) Crystal violet for five min, de-stained with sterile distilled water thrice, let to dry at room temperature, solubilized with 200 µL of 95% (v/v) ethanol for 10 min, and measured at 600 nm using ThermoFisher Scientific microplate reader.

Microplate biofilm assay for FTIR spectroscopy

Salmonella Typhimurium biofilm was grown in a 6-well microplate. Overnight inoculum (4 mL) was added to the microplate wells. Then, a volume of 1 mL of fresh nutrient medium was added. The microplate was incubated overnight at 37 °C. After 24 h period at 37 °C incubation, the content of the microplate was discarded while the microplate wells were rinsed with distilled water twice and the biofilm fraction was scrapped from the wall of the well after being suspended with phosphate-buffered saline. The suspension was then transferred into 1.5 mL centrifuge tubes and vortexed for 3 min. Then, they were centrifuged at 4000 rpm for 15 min at 4 °C to obtain the pellet. The resulting pellets were dried in the oven at 60 °C for at least 2 h.

FTIR spectroscopy

The biochemical composition of biofilm was determined using Perkin Elmer Spectrum One FTIR spectrometer. The dried cell pellets were positioned in direct contact with the diamond crystal, scanned in a range between 3000 cm^{-1} and 600 cm^{-1} with 4 cm^{-1} spectral resolution, and ratioed against a background spectrum previously collected from the clean sampling surface. Spectral data analysis, visualization, and processing were performed by using Perkin Elmer Applications Spectrum software.

Statistical analysis

Experimental data generated from crystal violet assay was expressed as mean ± standard deviation with n=3. A significant difference between control and test groups ($p<0.05$) was determined using an independent T-test.

RESULTS AND DISCUSSION

Figure 1 and 2 show the result of crystal violet assay. Treatment with all disinfectant significantly ($p<0.05$) reduced biomass of S. Typhimurium biofilm. Biofilm inhibition shown by MADIO PRO+, sodium hypochlorite, sodium dodecyl-benzene sulfonate, benzalkonium chloride and chloroxylenol were found to be 60.91%, 52.12%, 30.59%, 26.35%, and 58.07%, respectively. Treatment with MADIO PRO+ caused changes in FTIR spectral peaks associated with lipid (1460 cm^{-1}), protein (630 cm^{-1}, 702 cm^{-1}, 1550 cm^{-1}, 1650 cm^{-1}), and nucleic acid (1080 cm^{-1}, 1229 cm^{-1}). The biochemical modifications of S. Typhimurium biofilm were consistent with the inhibitory effects as shown by the crystal violet assay.

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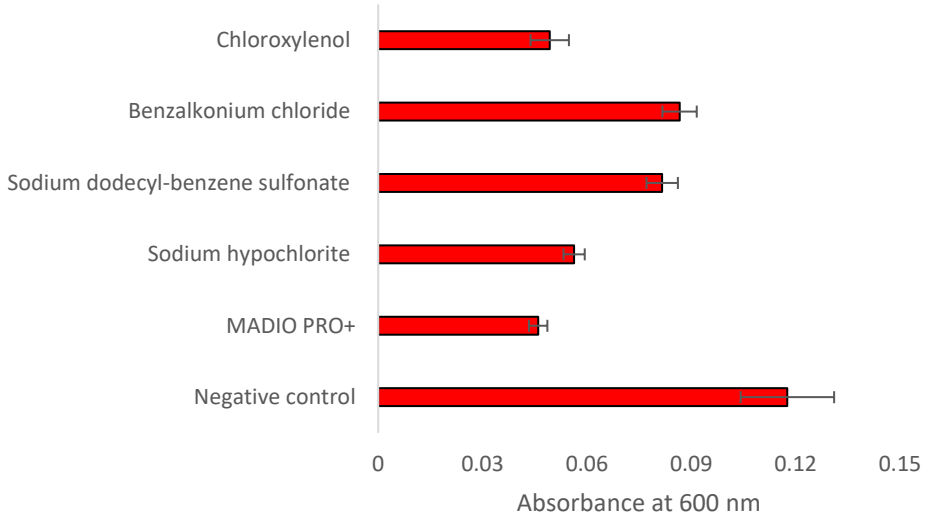

Figure 1. The effect of MADIO PRO+ multipurpose disinfectant on biomass of S. Typhimurium biofilm. This analysis was performed using crystal violet assay in 96-well microplate, n = 3. Significant difference between negative control and test sample is shown by *.

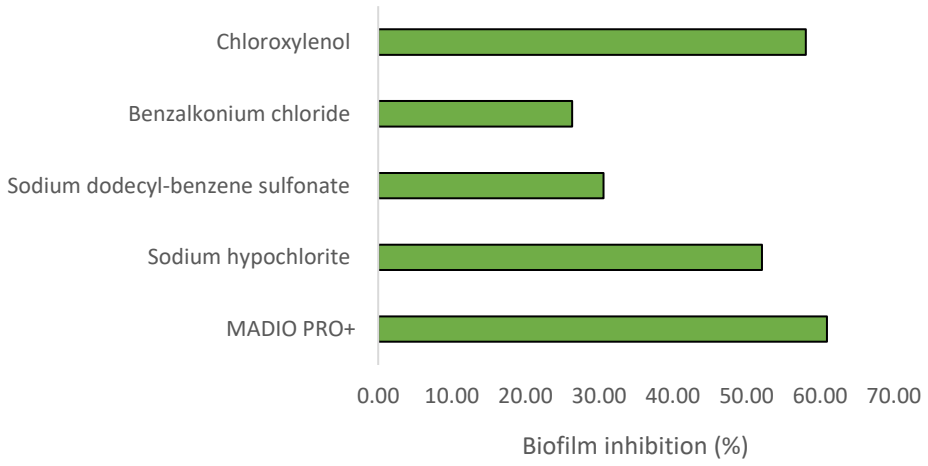

Figure 2. Biofilm inhibition by MADIO PRO+ multipurpose disinfectant. The mean absorbance values from the crystal violet assay were used to calculate the percentage inhibition of biofilm according to the following equation: Percentage (%) inhibition = (OD negative control - OD experimental) / (OD negative control) ×100.

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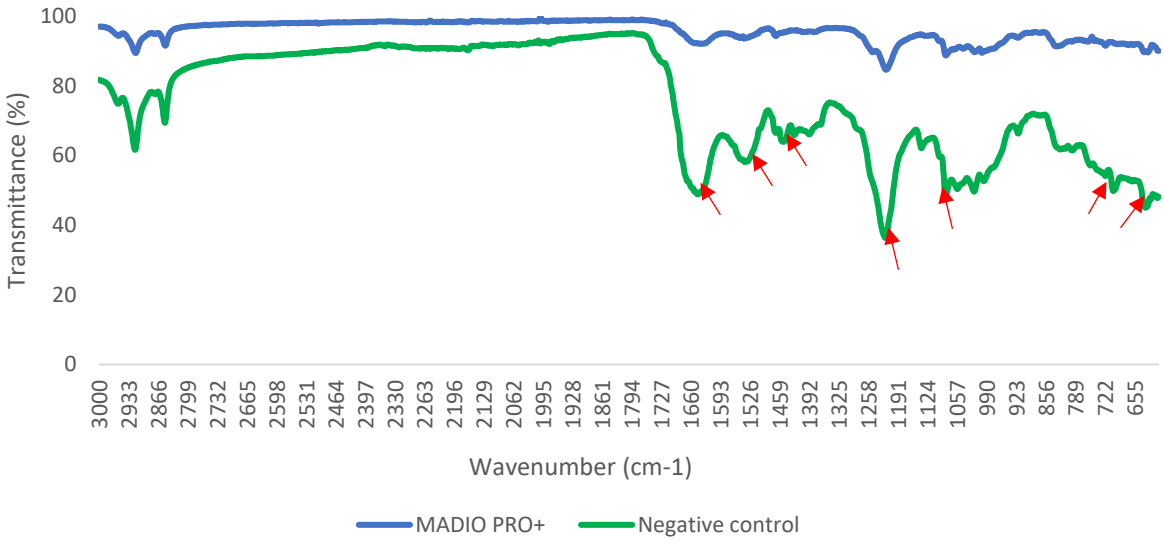

Figure 3. FTIR spectra of *S.* Typhimurium ATCC14028 biofilm treated with MADIO PRO+. Spectral regions showing organic molecules in the biofilm are in the range 600 – 3000 cm^{-1}. Negative control: bacterial inoculum with fresh broth. Arrows indicate spectral peaks undergoing changes.

Chloroxylenol, sodium dodecyl-benzene sulfonate, benzalkonium chloride, and sodium hypochlorite are disinfectants commonly used for disinfecting surfaces and cleaning equipments. They generally function by disrupting the bacterial cell membrane and interfering with cellular metabolism (Bhathal et al. 2018; Capita et al. 2019; Wang et al. 2016). In this study, MADIO PRO+ disinfectant effectively inhibited S. Typhimurium biofilm by modifying its structure. It is understood that changes in FTIR spectroscopic fingerprint, peak position, and peak intensity indicate the changes in the structure of biological molecules (DeQueiroz and Day 2007; Yahya et al. 2018; Kamaruzzaman et al. 2022).

CONCLUSION

The multipurpose disinfectant MADIO PRO+ has an advantage in controlling Salmonella biofilm. The structural changes in the Salmonella biofilm mediate its antibiofilm efficacy. This discovery could help the food and health care industries to plan better microbial intervention strategies.

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