

# Combined effects of UFJF\_PfSW6 phage and sodium hypochlorite for reducing *Pseudomonas* biofilm

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*Pseudomonas* are important spoilage bacteria in raw milk and dairy products. The persistence of these bacteria in the milk processing chain is due to their ability to form biofilms and high tolerance to disinfection processes. Bacteriophages, viruses that infect specifically bacteria, appear as one attractive solution for reducing or eradicating biofilms in the food industry. This study investigated the combined effects of UFJF\_PfSW6 phage and sodium hypochlorite in reducing *Pseudomonas fluorescens* biofilms formed on stainless steel surfaces at different temperatures. *P. fluorescens* UFV 041 was used to form biofilms on stainless steel coupons (AISI #304, 10mm x 10mm x 1mm) at 4 °C and 30 °C for 7 days utilizing UHT milk as the growth medium. Coupons containing biofilms were washed three times in 0.1% peptone water to remove non-adhered cells. Then, they were treated with bacteriophage suspension (10<sup>8</sup> PFU/mL) for 4 h and subsequently immersed in sodium hypochlorite at 100 ppm for 10 min. After disinfection, the coupons were transferred to tubes containing diluent with chlorine neutralizer (0.1% peptone water; 0.5% sodium thiosulphate) and then subjected to ultrasound (40 ± 2 kHz, 3 min) to remove any surviving adhered cells. The drop plate method estimated the bacterial counts and used untreated coupons as controls. Results were statistically analyzed using ANOVA and Scott-Knott test (P<0.05). All the treatments resulted in significant reductions of *P. fluorescens* (P<0.05) compared to the control, regardless of the temperature used for forming the biofilm. Individually, phage and sodium hypochlorite treatments reduced the bacterial count in biofilms formed at 30 °C by 0.9 and 0.6 log CFU/cm<sup>2</sup> and at 4 °C by 1.3 and 1.2 log CFU/cm<sup>2</sup>, respectively. On the other hand, higher reductions were achieved through the sequential application of phage and sodium hypochlorite, reaching values of 1.3 and 1.8 log CFU/cm<sup>2</sup> for biofilms formed at 30 °C and 4 °C, respectively. Our findings suggest that phages can be combined with chlorine to improve the removal of *P. fluorescens* biofilms, providing a potential alternative for controlling these bacteria in the food industry. However, further studies are necessary to determine the influence of other factors, such as phage and sanitizer concentrations, contact times, and disinfection temperatures, to achieve biofilm eradication.

**Key words:** Viruses; Anti-biofilm; Chlorine; Adhesion.

## Efeito combinado do fago UFJF\_PfSW6 e hipoclorito de sódio para redução de biofilmes de *Pseudomonas*

Este estudo avaliou o efeito combinado do fago UFJF\_PfSW6 e do hipoclorito de sódio na redução de biofilmes de *P. fluorescens* formados em aço inoxidável em diferentes temperaturas. A combinação do fago e sanitizante levou as maiores reduções na contagem bacteriana do que a aplicação individual, atingindo valores de redução de 1.3 e 1.8 log UFC/cm<sup>2</sup> para biofilmes formados a 30 °C e 4 °C, respectivamente. Nossos resultados sugerem que os fagos podem ser combinados com cloro para melhorar a remoção de biofilmes de *P. fluorescens*, fornecendo uma alternativa potencial para o controle dessas bactérias na indústria de alimentos.

**Palavras-chave:** Vírus; Anti-biofilme; Cloro; Adesão.

Acknowledge: This study was funded by the MilkFund research fund, a partnership between Inovaleite Group and Instituto Sua Ciência (ISC), as well as by the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) - Grant No. CAG 00146-22. The research was conducted at the Federal University of Juiz de Fora, and the bacterial strain (*P. fluorescens* UFV 041) was generously provided by the Food Microbiology Laboratory at the Department of Microbiology, Federal University of Viçosa.