Listeria monocytogenes biofilm prevention using Enterococcus faecium semi-purified bacteriocin

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Listeria monocytogenes is an opportunistic foodborne pathogen, a ubiquitous bacterium able to persist and adhere in food processing environment forming biofilms. However, lactic acid bacteria, beyond their beneficial applications, can produce an arsenal of antimicrobials, including bacteriocins. The aim of this study is to evaluate the prevention of biofilm formation of different serovars of L. monocytogenes by applying a semi-purified bacteriocin produced by Enterococcus faecium ST02JL, a strain isolated from artisanal cheesemaking environment. E. faecium ST02JL was cultured in MRS broth for 18h at 37ºC, and cell-free supernatant (CFS) was obtained by centrifugation (4000×g, 30 min, 4ºC), filter sterilized (0.22 µm), and heat-treated (80ºC for 10 min). Antimicrobial proteins were precipitated (60% ammonium sulfate saturation), collected by centrifugation (20000×g, 60 min, 4ºC), and re-suspended in 25 mM phosphate buffer (pH 6.5). Chromatography on SepPakC18 column was applied for further separation and targeted proteins eluted by 60% iso-propanol/25 mM phosphate buffer. For the biofilm inhibition study, 18h-old cultures of the studied L. monocytogenes strains were grown in TSBYE to a final cell concentration of ~107 CFU/mL. Each strain was transferred to 96-well microtiter plates and supplemented with semi-purified bacteriocin at different levels of activity. The biofilm challenge assay was performed at 25ºC for 48h in triplicates, where quantification of biofilms was made through Crystal Violet Assay. The semi-purified bacteriocin produced by E. faecium ST02JL strain promotes the biofilm inhibition of each evaluated L. monocytogenes strain in the treatment of at least 800 AU/mL bacteriocin. Considering that, a previous estimated MIC of ≥3200 AU/mL for the bacteriocin produced by E. faecium ST02JL, our observations confirmed the even sub-MIC bacteriocin activity has inhibitory effects against Listeria monocytogenes biofilm formation. Bacteriocins can be an alternative for controlling the biofilm formation in the industry surfaces.

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