Abstract

This study aimed to investigate the antibacterial and anti-biofilm activities of engineered *Salmonella* phage PVP-SE1 endolysin against *Salmonella* Typhimurium, *Escherichia coli*, *Staphylococcus aureus*, and *Listeria monocytogenes*. The antibacterial activity of the endolysin was also evaluated in high-and low-fat milk models stored at different temperatures (4, 25, and 37 ◦C). The engineered endolysin gene was expressed by induction with isopropyl β-D-1-thiogalactopyranoside (IPTG). Protein purification was carried out using affinity chromatography, and the purification of the engineered endolysin was confirmed by electrophoresis. The minimum inhibitory concentration (MIC) of the endolysin was determined using the microdilution method. The lytic activity of the endolysin against the studied bacteria was evaluated by turbidity reduction assay. The anti-biofilm activity of the engineered endolysin was assessed in culture medium and in the presence of organic compounds of milk, and the biofilm morphology was observed using scanning electron microscopy (SEM). The antibacterial activity of the engineered endolysin against *Salmonella* Typhimurium and *Listeria monocytogenes* in UHT milk was evaluated using culture and colony counting methods. Expression and purification of the engineered endolysin were confirmed by the observation of a 28 kDa band after vertical electrophoresis. Minimum inhibitory concentrations of the endolysin against *E. coli* and other bacteria were 250 and 500 μg/mL, respectively. The endolysin showed lytic activity against all bacteria. The time-kill assay revealed that the endolysin (500 μg/mL) could decrease the viable count of *S*. Typhimurium, *E. coli*, *S. aureus*, and *L*. *monocytogenes* by 2.23, 3.85, 1.89, and 1.48 log CFU/mL, respectively. The endolysin also reduced the biofilms of *S*. Typhimurium, *E. coli*, *S. aureus*, and *L*. *monocytogenes* within 2 h of exposure up to 1.84, 1.76, 1.31, and 1.05 log CFU/mL, respectively. These results were further confirmed by scanning electron microscopy. The endolysin showed higher antibacterial performance in low-fat milk than high-fat milk. Moreover, the endolysin was more effective at 25 ◦C and 37 ◦C than 4 ◦C. In conclusion, the endolysin could significantly reduce the planktonic and biofilm forms of food-borne pathogens, and also has potential to be used in milk to control food-borne pathogens.

Keywords: Antibacterial; endolysin; phage; biofilm; food; pathogen