# Abstract

Methicillin-resistant *Staphylococcus* spp. present challenges in clinical and veterinary settings because effective antimicrobial agents are limited. Phage-encoded peptidoglycan-degrading enzyme, endolysin, is expected to be a novel antimicrobial agent. The enzymatic activity has recently been shown to be influenced by the linker between functional domains in the enzyme. S6_ORF93 (ORF93) is one of the endolysins derived from previously isolated *Staphylococcus* giant phage S6. The ORF93 was speculated to have a catalytic and peptidoglycan-binding domain with a long linker. In this study, we examined the influence of linker shortening on the characteristics of ORF93. We produce wild-type ORF93 and the linker deletion mutants using an *Escherichia coli* expression system. These mutants were designated as ORF93-Δ05, ORF93-Δ10, ORF93-Δ15, and ORF93-Δ20, from which 5, 10, 15, and 20 amino acids were removed from the linker, respectively. Except for the ORF93-Δ20, ORF93 and its mutants were expressed as soluble proteins. Moreover, ORF93-Δ15 showed the highest yield and bacteriolytic activity, while the antimicrobial spectrum was homologous. The cold storage experiment showed a slight effect by the linker deletion. According to our results and other studies, linker investigations are crucial in endolysin development.