

The efficacy of bacteriophage-encoded endo-lysin relies on active residues: A molecular perspective

Supplementary Data:

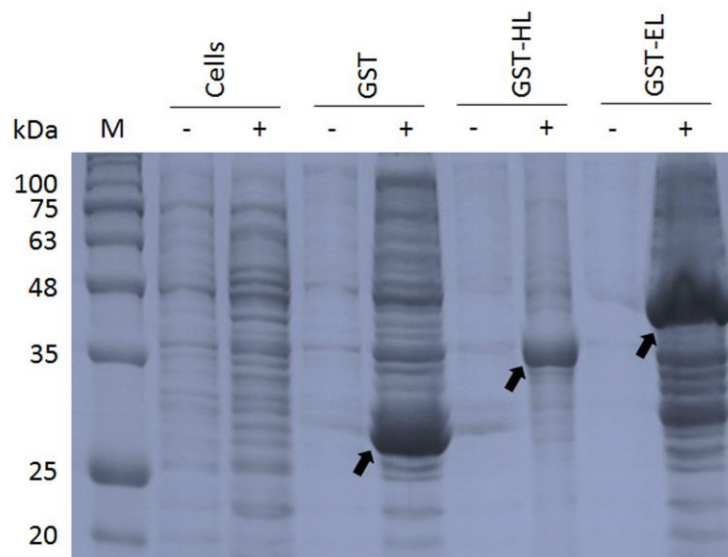


Figure 3A. *E. coli* BL21 (cells), BL21 cells with plasmid pGEX 4T-1 (GST, GST-HL and GST-EL) were expressed by the induction of IPTG, which indicated by a positive sign (+) and non-IPTG induced cells were indicated by a negative sign (-). M indicated Protein Marker. The arrows indicate the expressed protein glutathione s-transferase (GST) (~26 kDa) (Lane-5), GST-tagged holin (GST-HL) (37.7 kDa) (Lane-7) and GST-tagged endolysin (GST-EL) (43.4 kDa) (Lane-9).

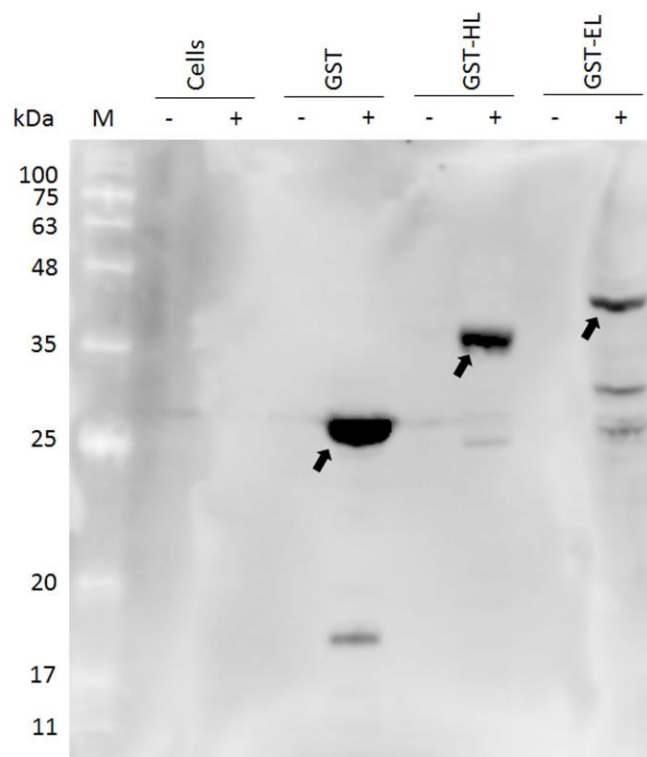


Figure 3B. *E. coli* BL21 (cells), BL21 cells with plasmid pGEX 4T-1 (GST, GST-HL and GST-EL) were expressed by the induction of IPTG, which indicated by a positive sign (+) and non-IPTG induced cells were indicated by a negative sign (-). M indicated Protein Marker. The arrows indicate the expressed protein glutathione s-transferase (GST) (~26 kDa) (Lane-5), GST-tagged holin (GST-HL) (37.7 kDa) (Lane-7) and GST-tagged endolysin (GST-EL) (43.4 kDa) (Lane-9). Protein samples in the supernatant were assessed by western blot analysis with an anti-GST antibody.

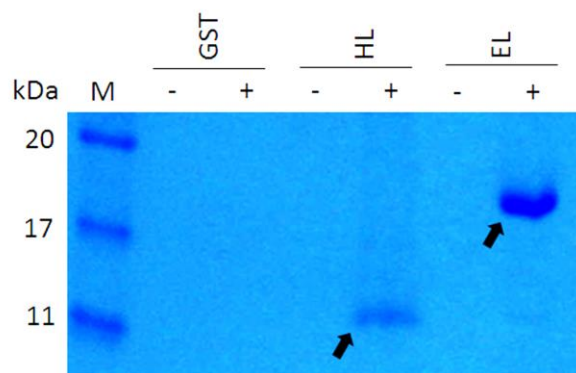


Figure 3C. Purification of Holins and Endolysins. The GST tag was eluted by thrombin after the expression of GST-HL and GST-EL. Arrows indicate purified holins (11.7 kDa)(Lane-5) and endolysins (17.4 kDa)(Lane-7) by SDS–PAGE. Protein samples were expressed by the induction of IPTG, which indicated by a positive sign (+) and non-IPTG induced cells were indicated by a negative sign (-). M indicated Protein Marker.

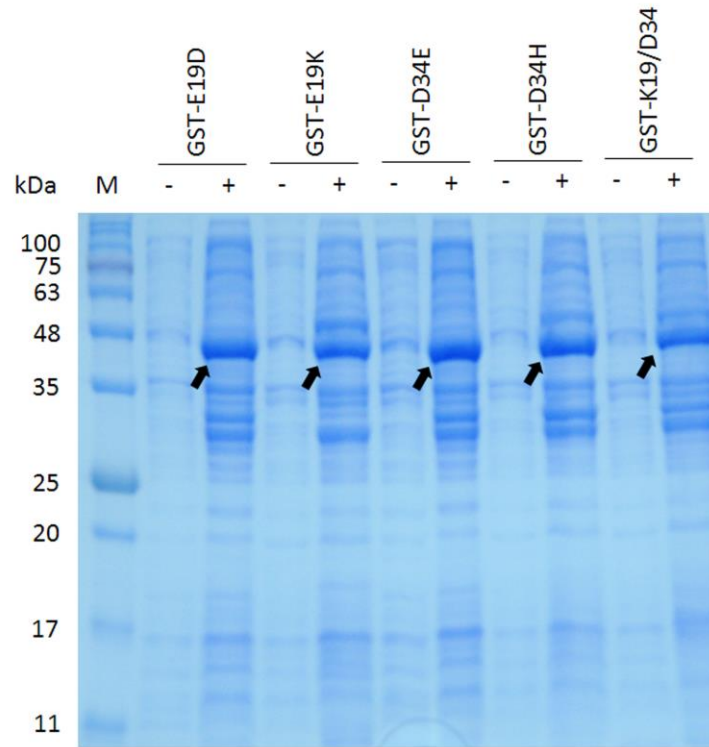


Figure 4B. Endolysin mutants expression confirmed by SDS-PAGE. All five endolysin mutants (GST-E19D, GST-E19K, GST-D34E, GST-D34H and GST-K19/H34) (~43.4 kDa) were assessed by SDS-PAGE analysis. Each sample contains a control, indicated by a negative sign (-). The arrows indicate the expressed protein GST-tagged endolysins mutants (43.4 kDa). M indicated Protein Marker.

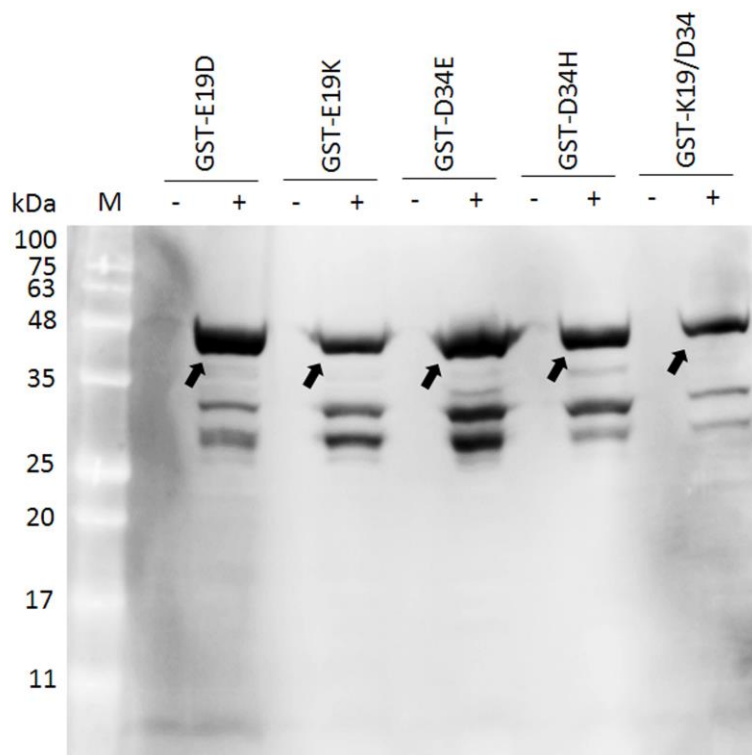


Figure 4C. Endolysin mutants expression confirmed by western blot. All five endolysin mutants (GST-E19D, GST-E19K, GST-D34E, GST-D34H and GST-K19/H34) (~43.4 kDa) were assessed by western blot analysis with an anti-GST antibody. Each sample contains a control, indicated by a negative sign (-). The arrows indicate the expressed protein GST-tagged endolysins mutants (43.4 kDa). M indicated Protein Marker.

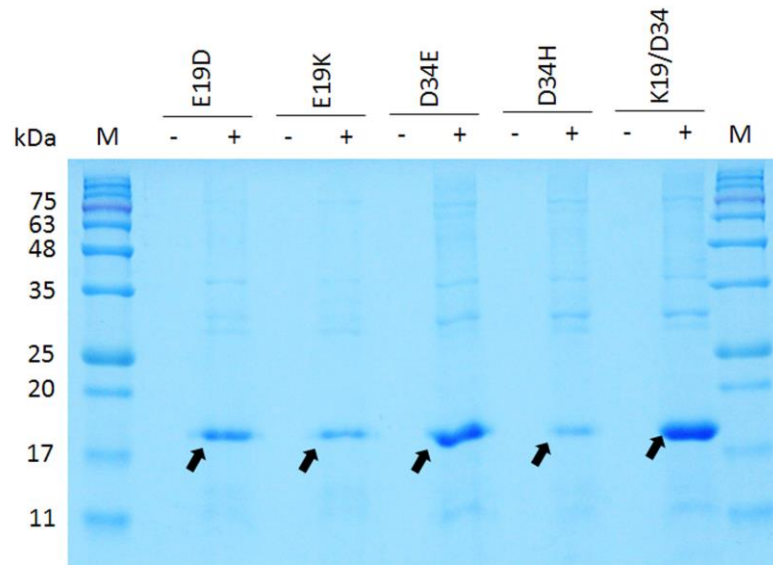


Figure 4D. Pure endolysin mutants expression confirmed by SDS-PAGE. The GST tag was eluted by thrombin and pure endolysin mutants were confirmed by SDS-PAGE with protein marker (M). The arrows indicate the expressed protein pure endolysins mutants (~17.4 kDa). Each sample contains a control, indicated by a negative sign (-).