

Supplementary Material

Novel signal peptides improve the secretion of recombinant *Staphylococcus aureus* Alpha toxin_{H35L} in *Escherichia coli*.

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Table S1. Identified peptide sequences of secreted AT_{H35L} in culture medium

The position of amino acid	Released AT _{H35L} from NSP4	AT _{H35L}
1	A	A
2	D	D
3	S	S
4	D	D
5	I	I
6	N	N
7	I	I
8	K	K
9	T	T

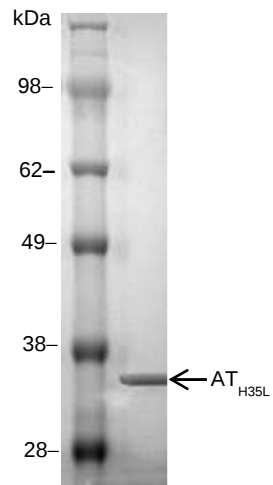


Fig S1. SDS-PAGE analysis of purified AT_{H35L} protein. Secreted AT_{H35L} by NSP4 in culture medium was purified and used for N-terminal peptide sequencing analysis.

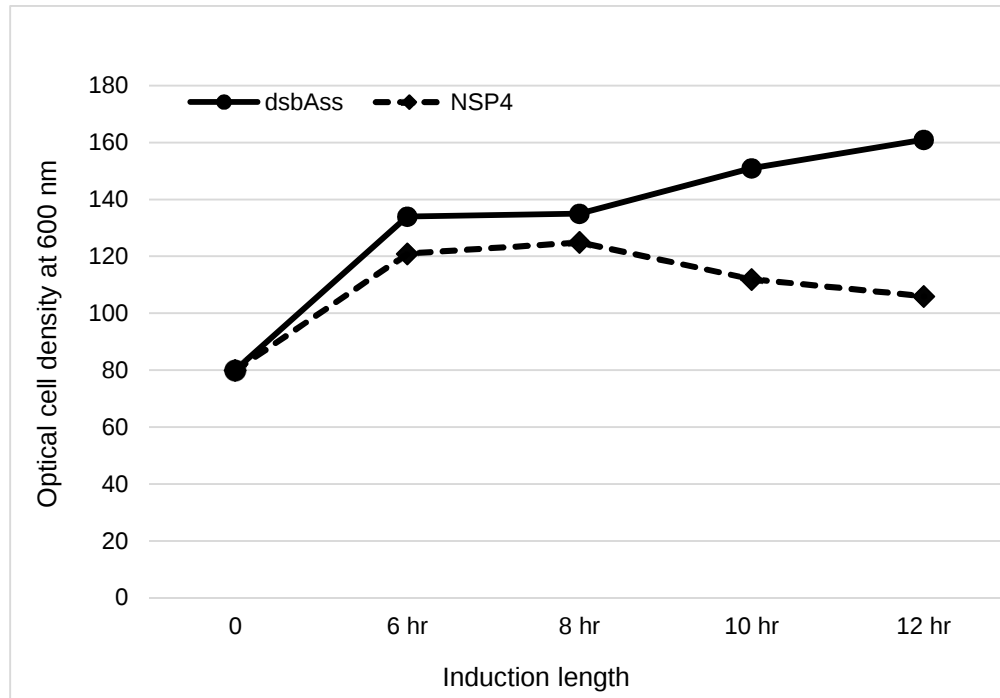


Fig. S2 The cell growth profiles of NSP4_AT_{H35L} and dsbAss_AT_{H35L}. Recombinant AT_{H35L} gene expression was induced by addition of 0.5 mM IPTG at an optical cell density of 80 (OD₆₀₀). After induction, cell culture was continued in fed-batch mode at 30 °C for an additional 12 h. Cultured cells were collected at different time points post-induction to determine the profiles of secreted AT_{H35L} protein in culture medium.

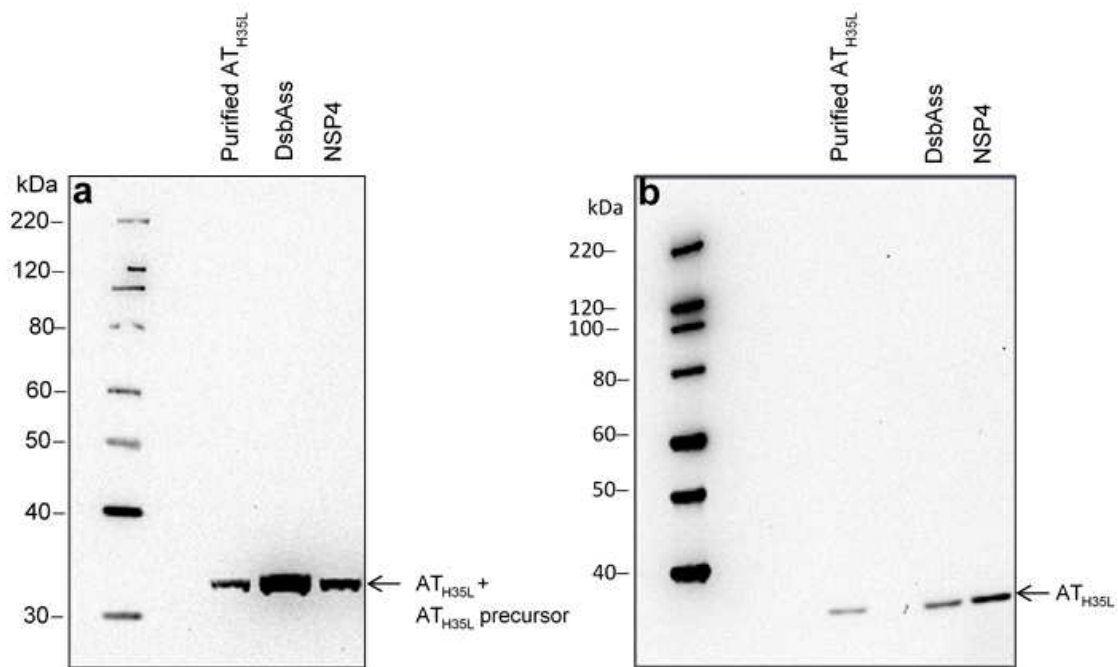


Fig S3. Western blot analysis of cell lysates and cell culture medium from *dsbA_AT_{H35L}* and *NSP4_AT_{H35L}* in a Micro24 fed-batch process. (a) Arrow indicates AT_{H35L} protein in cell crude lysates. (b) Arrow indicates released mature AT_{H35L} protein in cell culture medium. Purified AT_{H35L} protein was used as a reference.