Supplementary Material

An RNA-helicase important for *Listeria monocytogenes* haemolytic activity and virulence factor expression

Sakura Netterling[1,2,3], Caroline Bäreclev[1,2,3], Karolis Vaitkevicius[1,2,3] and Jörgen Johansson[1,2,3*]

[1]Department of Molecular Biology

[2]Molecular Infection Medicine, Sweden (MIMS)

[3]Umeå Center for Microbial Research (UCMR)

Umeå University, Sweden

*Corresponding author: Jorgen.johansson@molbiol.umu.se

Figure S1

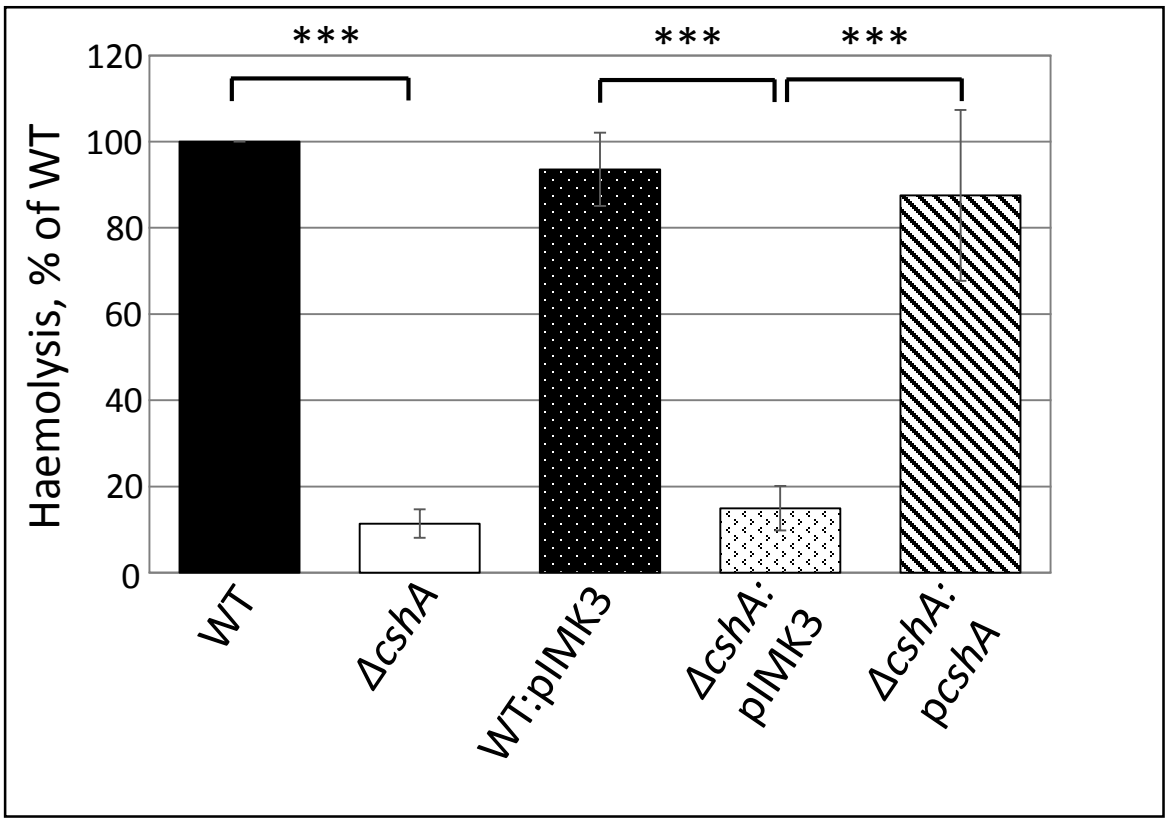


Figure S1. *Haemolytic activity of indicated strains*. Indicated strains were grown at 37 °C in pH-adjusted BHI (pH 5.5, +IPTG 1mM) until an O.D.600 ≅ 0.9., before supernatant were filtered and added to an 1:1 ratio to 10 % suspension of red blood cells and incubated for 3 hours at 37 °C. The absorbance was measured at 541 nm. The figure shows the hemolytic activity in percentage relative to wild-type (100%). All samples were compared with the wild-type or Δ*lmo0866*:pIMK3 (three last bars) using Student T-test (two-tailed) ($p<0.001$, ***).

Figure S2

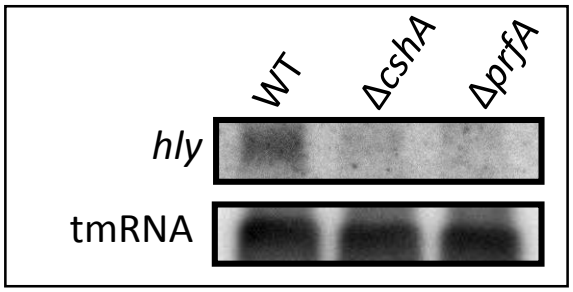
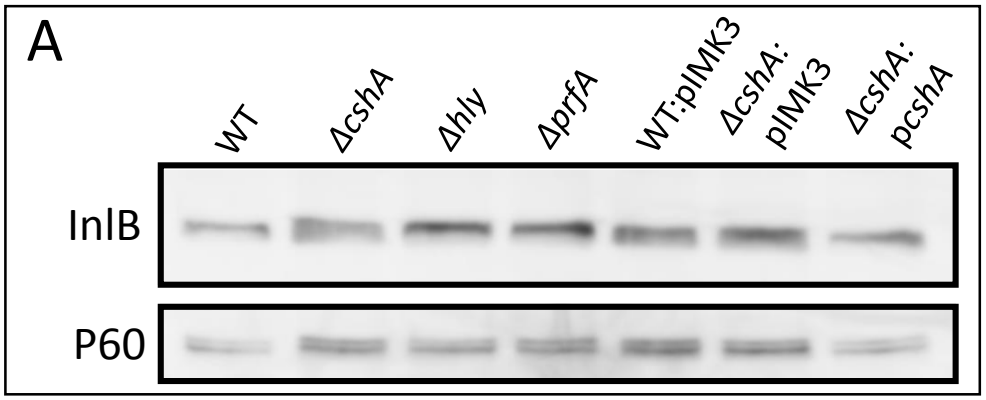
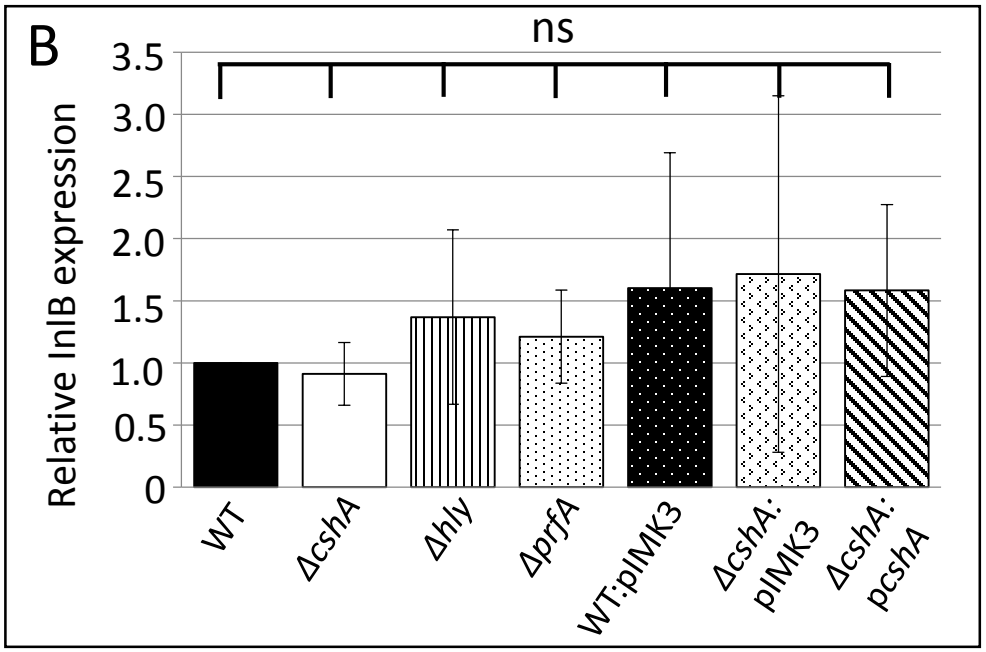


Figure S2. *Northern blot.* Indicated strains were grown at 37°C in pH-adjusted BHI (pH 5.5) until an $O.D._{600} \cong 0.9$., before RNA isolation and separation. Expression of *hly* and tmRNA (control) transcripts was analyzed by Northern blotting of RNA isolated from wild-type, *Δlmo0866* or Δ*prfA* strains, respectively.

Figure S3





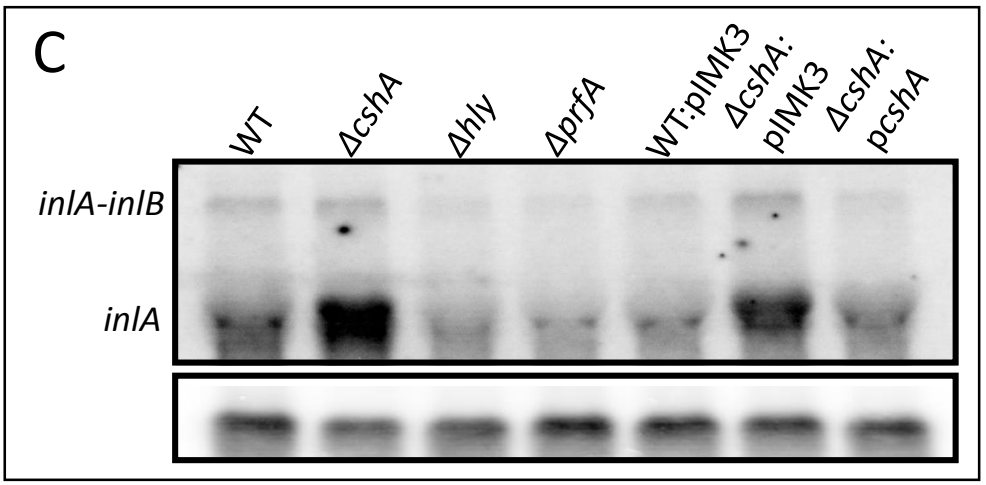


Figure S3. *Adhesin expression in different strain backgrounds*. **A.** The indicated strains were grown at 37°C in pH-adjusted BHI (pH 5.5) until an OD_{600} ≅ 0.9 before protein extraction, SDS-PAGE separation and Western blot analysis. Expression levels of InlB (upper panel) or P60 (control, lower panel) were analyzed, using InlB- or P60-specific antibodies, respectively. n=3. **B.** InlB expression (from A) was quantified in the indicated strains and related to P60 expression. InlB expression in EGDe (WT) was arbitrarily set to 1.0. Error bars show standard deviation. For statistics, all samples were compared with EGDe using Student's T-test (two-tailed) ns = not significant. **C.** Northern blot of *inlA* and *inlB* expression. Indicated strains were grown at 37°C in pH-adjusted BHI (pH 5.5) until an $O.D._{600}$ ≅ 0.9., before RNA isolation and separation. Expression of *inlA-inlB* bicistronic messenger and *inlA* (upper panel) or tmRNA (lower panel, control) was analyzed by Northern blotting of RNA using radioactively labelled DNA-probes specifically recognizing *inlA/inlB* or tmRNA respectively.