SUPPLEMENTARY MATERIAL

Immunogenicity and protection by DnaK and SpaA recombinant proteins against Erysipelothrix rhusiopathiae in a murine model

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Materials and Methods

1. Heterologous expression of rDnaK and rSpaA in ClearColi

The recombinant vectors containing the coding sequences for the DnaK and SpaA proteins were transformed by electroporation into the *Escherichia coli* electrocompetent ClearColi™ BL21(DE3) (Lucigen) for endotoxin-free protein production, and transformants were selected in solid medium Luria Bertani (LB) from Sigma-Aldrich, containing 30 ug/mL kanamycin. DnaK (rDnaK) and SpaA (rSpaA) were respectively expressed from an isolated colony of each transformant (ClearColi pET28a+DnaK and ClearColi pET28a+SpaA) in 5 mL of media Terrific Broth (TB) (20 g/L tryptone; 24 g/L yeast extract; 17 mM KH_2PO_4; 72 mM K_2HPO_4; 0.4% glycerol) and LB media. An aliquot of 2.5 mL volume was inoculated in 250 mL culture, and maintained at 37 °C and 250 rpm until reach exponential phase, when expression was induced with isopropyl β-D-1-thiogalactopyranoside (IPTG) at 0.1 mM for 18 hours at 20 °C and 250 rpm.

2. Sonication of cultured bacteria

Cultures were centrifuged 10,000 x *g* for 15 minutes at 4 °C and the cells were suspended in 20 mL Tris-NaCl sonication buffer (50 mM Tris-HCl, 250 mM NaCl, pH 8), containing 0.1 mM phenylmethylsulfonyl fluoride (PMSF). After sonication with 16 pulses of 23% amplitude for 30 seconds, with a 30-second interval between pulses using a Sonic Dismembrator Model 500 (Fisher Scientific), lysates were centrifuged under the same conditions to separate supernatant (soluble) and pellet (insoluble) fractions.

Figures

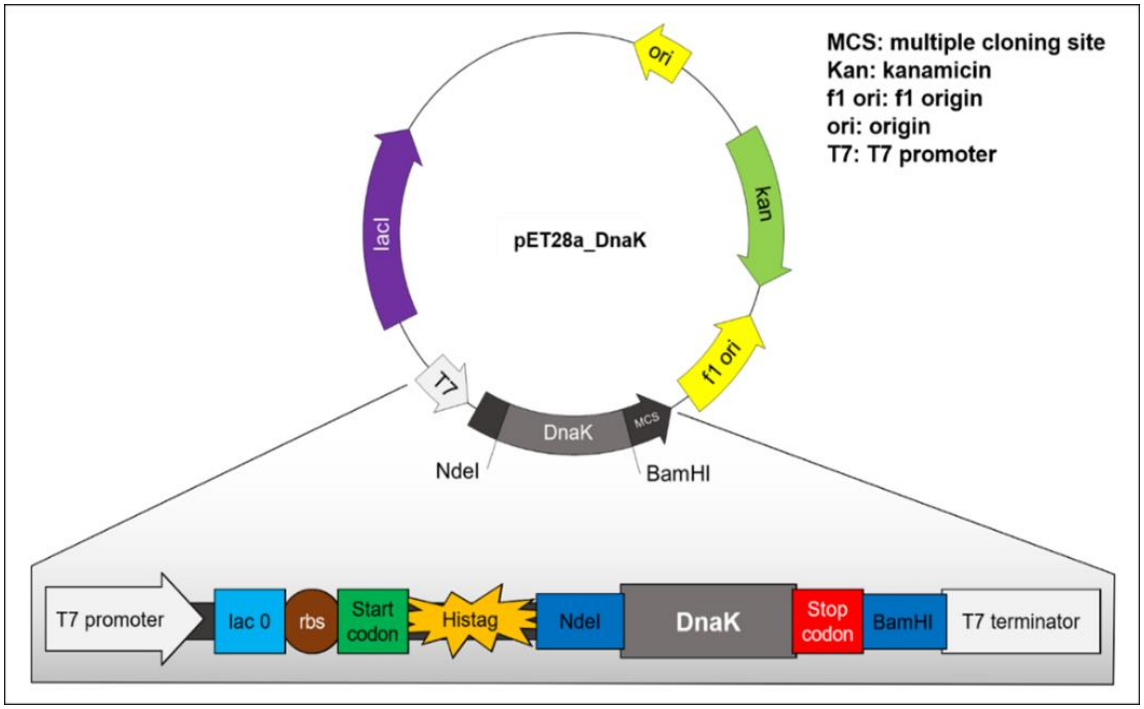

Figure S1: Construction pET28a_DnaK. Simplified circular map of the pET28a_DnaK construct and linear map of the cloning sequence, indicating the location of the 6 histidine tail and the restriction sites.

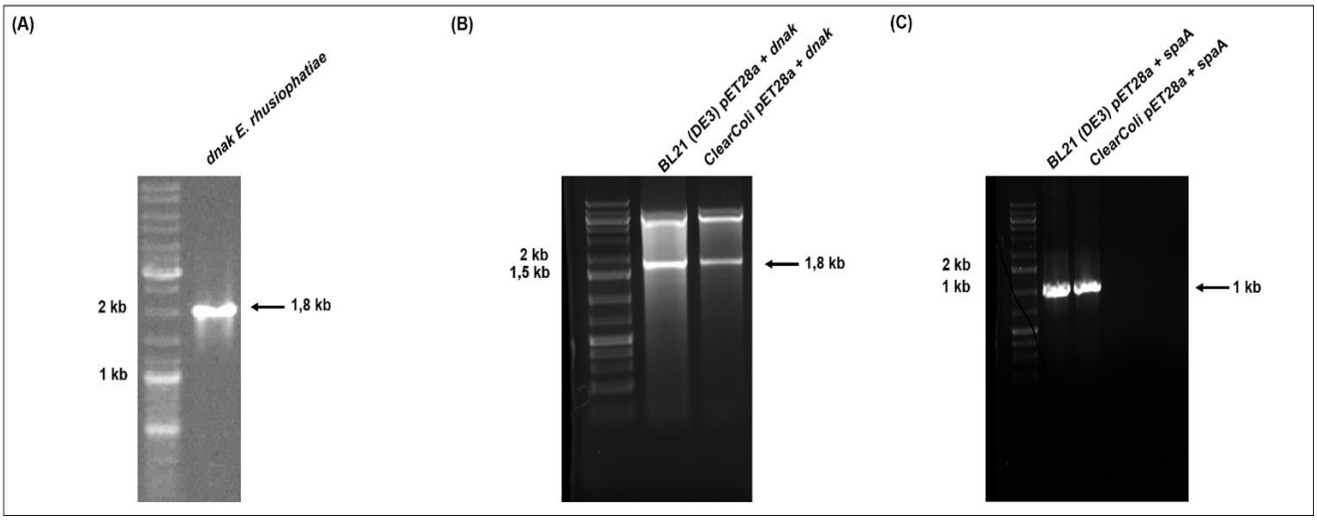

Figure S2: Amplification of the *dnak* gene and confirmation of transformation of clones by electroporation in ClearColi. (A) Amplification of the coding sequence of DnaK, from *E. rhusiopathiae* NCTC11002, by primers DnaK_For_NdeI and DnaK_Rev_BamHI. (B) Fragment *dnak* cloned into plasmid pET28a, from a mini-prep of *E.coli* bacteria BL21(DE3) and ClearColi digested with restriction enzymes *Bam*HI and *Nde*I. (C) Fragment *spaA* cloned into plasmid

pET28a, after mini-prep of *E.coli* BL21(DE3) and ClearColi bacteria and amplification with primers SpaA_For-NcoI and SpaA_Rev-HindIII.

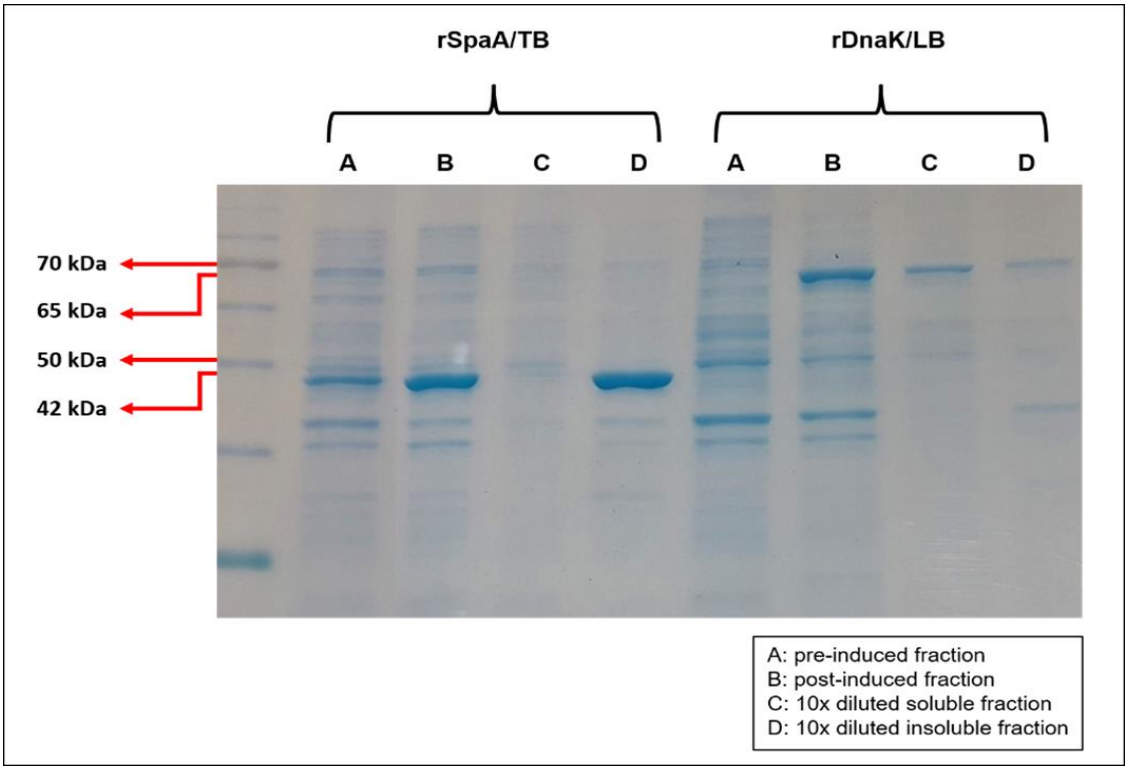

Figure S3: Expression of rSpaA and rDnaK recombinant proteins in ClearColi. The rSpaA protein has a size of 42 kDa on the gel and was induced in TB, while the rDnaK has a size of 65 kDa and was induced in LB culture medium. For the expression of the two proteins, a temperature of 20°C and 0.1 mM of IPTG was used. (A) Fraction before IPTG addition. (B) Fraction after IPTG addition in the culture medium, showing the induction of expression of recombinant proteins. After being induced, the cultured cells were lysed by ultrasound and separated into soluble and insoluble fractions, with rDnaK being found in the soluble fraction (C) and to a lesser extent in the insoluble fraction (D) and rSpaA only in the insoluble fraction (D).

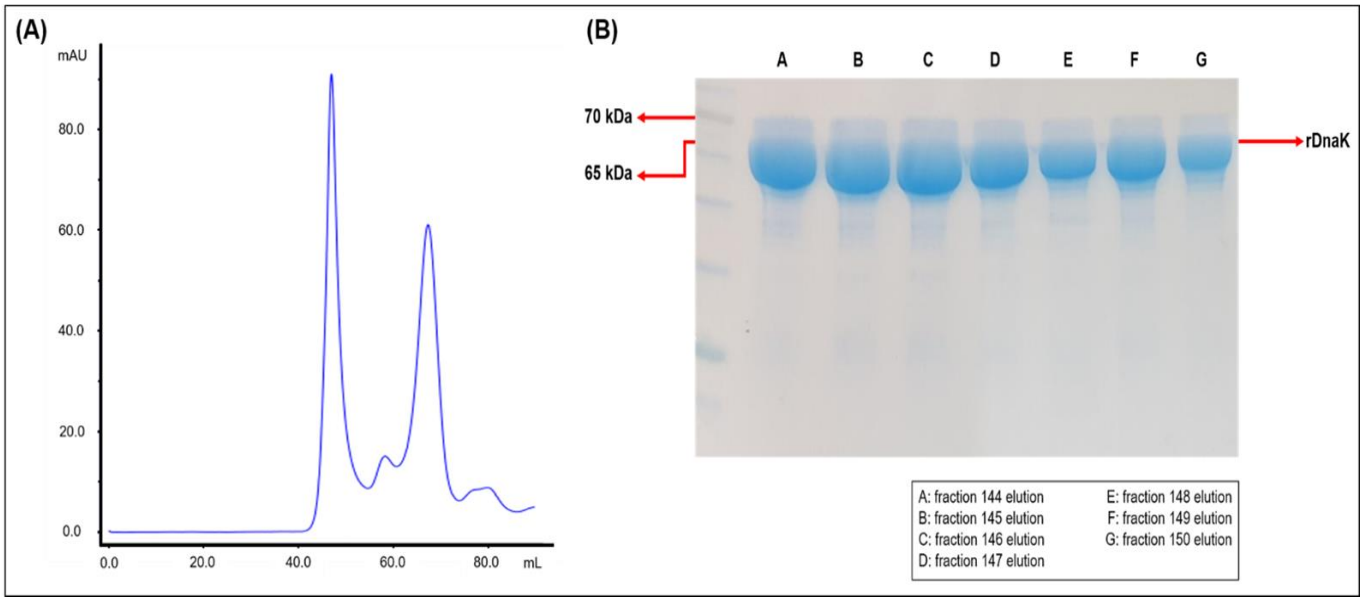

Figure S4: SDS-PAGE analysis of molecular exclusion chromatography of rDnaK. (A) Chromatogram of the purification of rDnaK in which the protein was eluted at the third peak at an absorbance of 60 nm (B) After purification on a nickel column, the rDnaK protein was subjected to molecular exclusion chromatography and then eluted mainly in fractions 144 to 150, which correspond to a peak formed in the chromatogram. The fractions correspond to the expected size of the rDnaK protein (65 KDa). Note: protein concentrations were not quantified or equalized for the analysis of this gel.

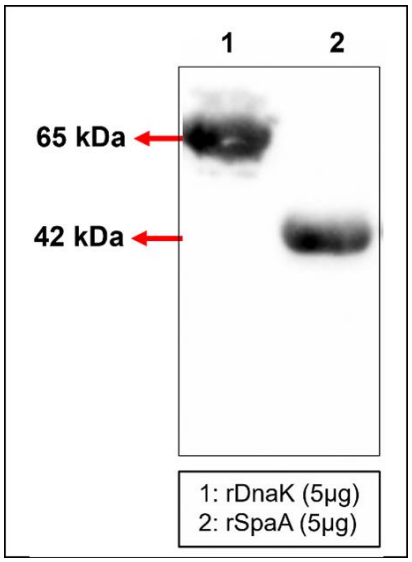

Figure S5: Western Blot of *E. rhusiopathiae* SpaA and DnaK recombinant proteins with anti-*HisTag* mouse monoclonal antibody. The bands show the reaction of the histidine tag, present in purified and equalized 5 µg of rDnaK (1) or rSpaA (2) proteins, against *anti-HisTag* antibodies. The two bands correspond to the expected sizes for the two recombinant proteins.