Supplementary Information

Generation of mutant *P. multocida*

P.multocida serotype B:2 was confirmed via species-specific polymerase chain reaction (PCR) using primer sets KMT1T7/KMT1SP6 and CAPB-F/CAPB-R, yielding expected amplicons of 460 bp and 750 bp, respectively (Fig. S2a).

For the targeted deoptimization of the *fis* gene, primers FLN1 and FLN2 were employed to amplify a 2,357 bp DNA fragment comprising the 300 bp *fis* gene flanked by approximately 1 kb of upstream and downstream regions (Fig. S2b). This fragment included XbaI and SacI restriction sites within the flanking regions, allowing for its ligation into the pDS132 vector digested with the same enzymes. The resultant construct, pDS132F, was confirmed via double digestion, revealing a 2,357 bp insert (Fig. S2c).

To facilitate deoptimized expression of *fis*, the whole 300 bp of *fis* gene was recoded based on the codon usage of *P. multocida* (Kazusa Database, Taxonomy ID: 272843), and synthesized with 231 bp upstream and 29 bp downstream unmodified flanking regions (Fig. S1). The recoded sequence was custom synthesize by vendor (GenScript). The vendor supplied us synthetic gene cloned into pUC57 vector, the resulting plasmid is named as pUC57FS.

Subsequently, the *fis* gene in pDS132F was replaced with the recoded version through double digestion of both pDS132F and pUC57FS (Fig. S2d) using MssI and SpeI enzymes. The 560 bp recoded insert was gel-purified and ligated into the vector backbone, producing the recombinant construct pDS132FM (Fig. S2e). This construct was verified via restriction digestion. The sequence integrity and orientation of the inserted gene were confirmed by commercial sequencing (Bioserve, India).

Electrocompetent *P. multocida* cells were transformed with pDS132FM via electroporation and selected on BHI agar plates containing 30 μg/mL chloramphenicol at 37°C. Chloramphenicol-resistant colonies indicated successful single crossover events. For double crossover selection, colonies were screened on LB agar supplemented with 10% sucrose. Among 192 sucrose-resistant colonies, 35 were chloramphenicol-sensitive, and only one colony tested positive for the presence of the recoded *fis* gene using two set of primer. PCR primers (FSC1- FSC2) yielding 171 bp (Fig. S1f) product and PCR primers (FSC3- FSC2) yielding 196 bp product (Fig. S2g) and Sequencing of this PCR product confirmed the successful integration of the recoded gene into the genome of *P. multocida* in the correct orientation.

Recombinant Expression and Characterization of *fis* Protein in *E. coli*

The *fis* gene was amplified using PCR with specific primers (FEP1 and FEP2) producing and subsequently digested with *EcoRI* and *HindIII* restriction enzymes. The digested fragment was gel-purified and ligated into the pET32a(+) expression vector, which had been digested with the same enzymes. The recombinant plasmid was then transformed into *Escherichia coli* DH5α, and transformants were selected on LB agar containing ampicillin. Plasmid screening by restriction digestion confirmed the presence of a 303 bp insert (Fig. S3a), and sequencing verified the correct reading frame. BLAST analysis confirmed 100% identity with published *fis* gene sequences.

Following confirmation, the recombinant plasmid was introduced into *E. coli* BL21 cells for protein expression. Cultures were grown to mid-log phase and induced with varying concentrations of IPTG. The expressed *fis* protein, fused with a polyhistidine tag, was purified using nickel-affinity chromatography. SDS-PAGE analysis of the eluted fraction revealed a prominent band of approximately 29 kDa, indicating successful purification (Fig. S3b).

Western blotting using a Ni-NTA–HRP conjugate confirmed the identity of the protein, showing a specific immunoreactive band at the expected molecular weight (29 kDa), which includes both the *fis* protein and the fusion tag. To assess immunogenicity, mice were immunized with 25 μg of the purified recombinant protein through four subcutaneous injections over 28 days. Further validation using immunoblotting and hyperimmune serum at a 1:100 dilution revealed a clear 29 kDa band, confirming antigenic recognition of the recombinant *fis* protein by the murine immune system.

Table 1S: List of Primer Used

Name	**Sequence (5'-3')**	**RE Site**	Accession no-AE004439.1 and position
KMT1T7	ATCCGCTATTTACCCAGTGG		Townsend *et al*., 1998
KMT1SP6	GCTGTAAACGAACTCGCCAC		
CAPB-F	CATTTATCCAAGCTCCACC		Townsend *et al*., 2001
CAPB-R	GCCCGAGAGTTTCAATCC		
FLN1	AGCTATCTAGATCACCGCCGCGTTATCTTTA	XbaI	1281328-1281347
FLN2	ATCGAGAGCTCCTTATCGGTCCGGGTATGTT	SacI	1283682-1283663
FSC1	TCTGGTTTCCCCGCGTGTAC*		
FSC2	TCCCAGTCCCAGGTCACGAA*		
FSC3	TGGTGTGGAGAAAGGATGTCGT		1282811- 1282790
FEP1	TAGCGAATTCGAACAACAACGTAATCCTGC	EcoRI	1282616-1282635
FEP2	TAGCAAACTTAATGGTCCGTTAACCCATAC	Hind III	1282333- 1282352

***Primer design from synthetic depotmzed sequence**

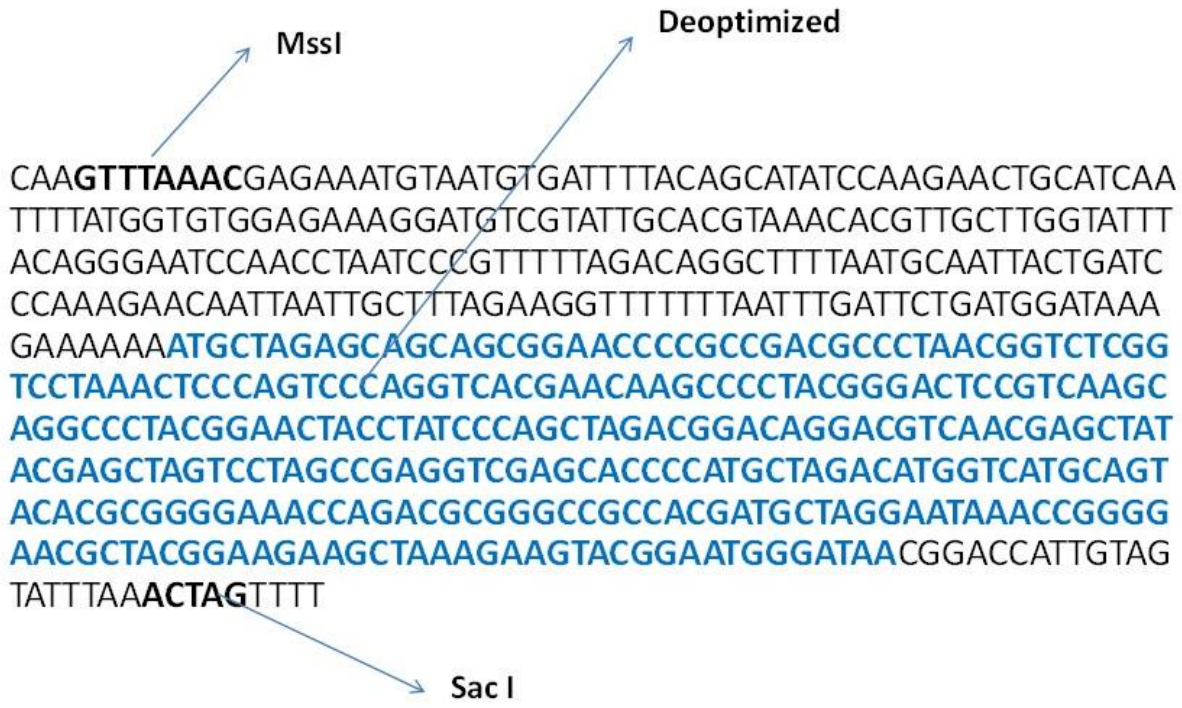

Fig1S: The nucleotide sequence of Synthetic Codon Deoptimized fis gene

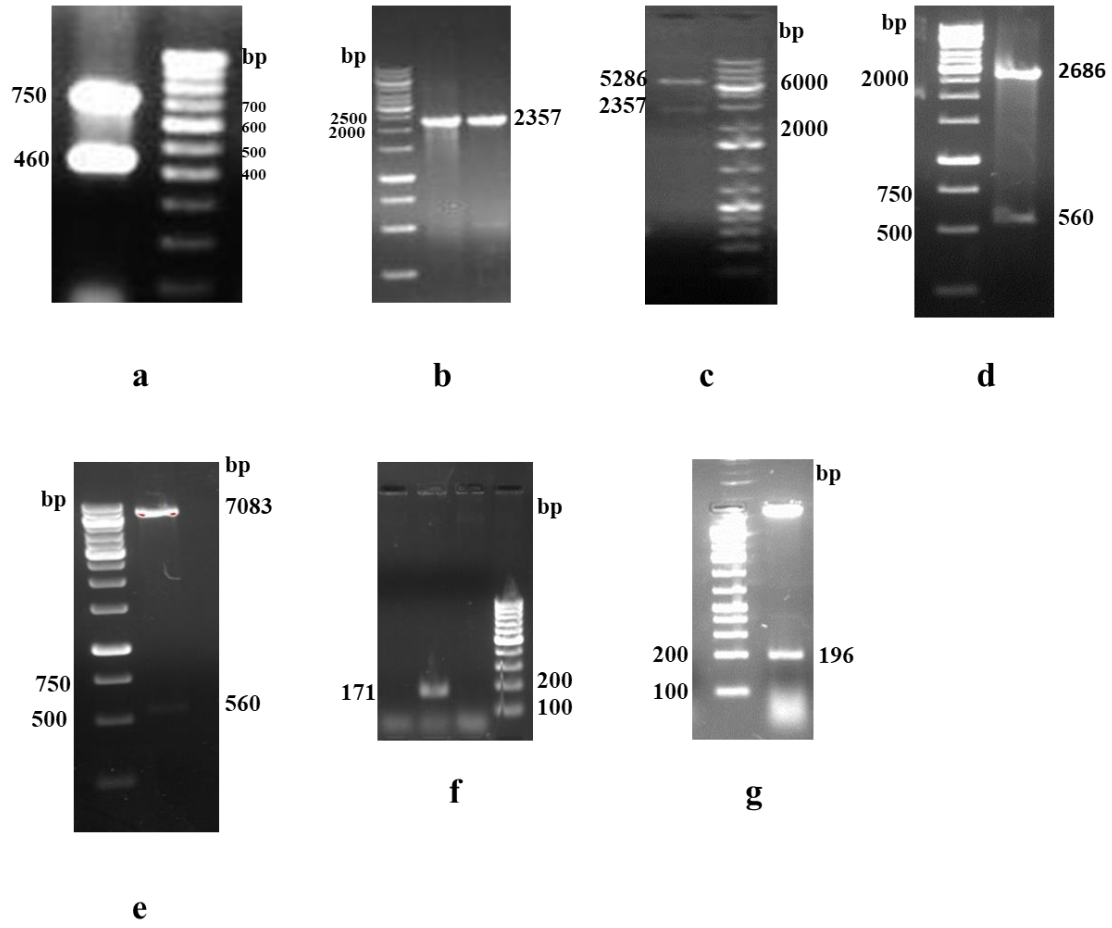

Fig 2S: Generation of mutant *P. multocida*

a. PCR confirmation of *P. multocida* using KMT1T7–KMT1SP6 and CAPB-F–CAPB-R primers, yielding expected amplicons of 460 bp and 750 bp, respectively.
b. Amplification of a 2,357 bp fragment comprising the 300 bp *fis* gene flanked by ~1 kb upstream and downstream regions using FLN1–FLN2 primers.
c. Digestion of the pDS132F vector containing the 2,357 bp DNA fragment with XbaI and SacI restriction enzymes.
d. Digestion of the pUC57FS vector containing a recoded 560 bp sequence with SpeI and MssI.
e. Digestion of the pDS132FM vector containing the 560 bp recoded sequence with SpeI and MssI.
f. PCR confirmation of mutant *P. multocida* using FSC1–FSC2 primers.
g. PCR confirmation of mutant *P. multocida* using FSC3–FSC2 primers.

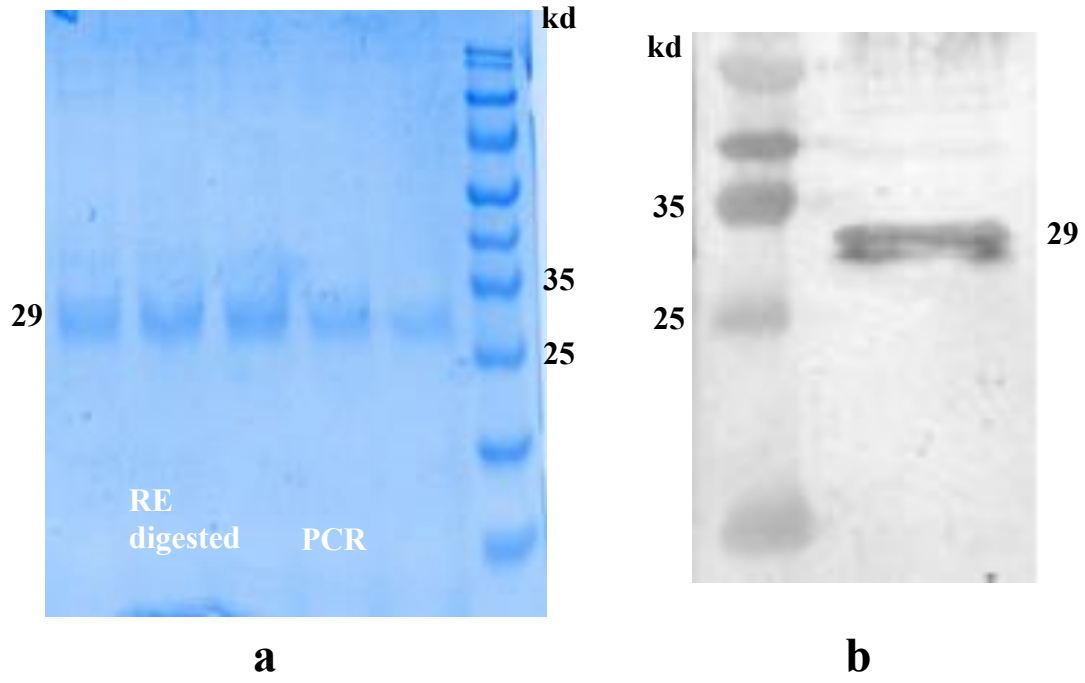

Fig S3: **Recombinant Expression and Characterization of *fis* Protein in *E. coli***

a. SDS-PAGE analysis of the expressed fis protein revealed a prominent band at approximately 29 kDa, corresponding to the expected size of the recombinant fusion protein.
b. Immunoblotting of the recombinant fis protein using hyperimmune serum confirmed its expression and antigenic identity.