Supplementary Material

Table S1. Strains and plasmids.

Strain	Description	Reference
B. bronchiseptica		
RB50	Wild type strain: RB50	(Cotter and Miller, 1994)
*Bb*WT	Wild type strain; 9.73H+ Sm^r	(Le Blay *et al.*, 1997)
BbWT-brtA-HA	*Bb*9.73H+ with *brtA* with HA tagg Sm^r	(Ambrosis *et al.*, 2016)
BbΔfhaB	*Bb*9.73H+ with *fhaB* deleted Sm^r	This work
BbΔdegP	*Bb*9.73H+ with *degP* deleted Sm^r	This work
BbΔcyaA	*Bb*9.73H+ with *cyaA* deleted Sm^r	This work
BbΔcyaAΔdegP	*Bb*9.73H+ with *cyaA* and *degP* deleted	This work
BbΔbvgR	*Bb*9.73H+ with *bvgR* deleted Sm^r	(Gutierrez et al., 2024)
BbΔpdeD	*Bb*9.73H+ with *pdeD* deleted Sm^r	(Gutierrez et al., 2024)
BbΔpdeA	*Bb*9.73H+ with *pdeA* deleted Sm^r	This work
BbΔpdeC	*Bb*9.73H+ with *pdeC* deleted Sm^r	This work
*Bb*Δ3PDE	*Bb*9.73H+ with *pdeA, pdeC,* and *pdeD* deleted Sm^r	This work
*Bb*Δ4PDE	*Bb*9.73H+ with *pdeA, pdeC, pdeD* and *bvgR* deleted Sm^r	This work
BbΔlapG	*Bb*9.73H+ with *lapG* deleted Sm^r	(Ambrosis *et al.*, 2016)
BbΔbrtA	*Bb*9.73H+ with *brtA* deleted Sm^r	(Ambrosis *et al.*, 2016)
Plasmids		
pMQ30	allelic replacement; *sacB aacC1* ColE1 *oriT* CEN4 URA3 Gm^r	(Shanks *et al.*, 2006)
pMQ30*pdeA* F1F2	pMQ30 containing *pdeA* upstream and downstream region Gm^r	This work
pMQ30*pdeC* F1F2	pMQ30 containing *pdeC* upstream and downstream region Gm^r	This work
pMQ30*pdeD*F1F2	pMQ30 containing *pdeD* upstream and downstream region Gm^r	(Gutierrez *et al.*, 2024)
pMQ30*fhaB* F1F2	pMQ30 containing *fhaB* upstream and downstream region Gm^r	This work
pMQ30*degP* F1F2	pMQ30 containing *degP* upstream and downstream region Gm^r	This work
pMQ30*cyA* F1F2	pMQ30 containing *cyaA* upstream and downstream region Gm^r	This work
pEmpty	pBBR1-MCS-5-*npt*II Gm^r	(Sisti *et al.*, 2013)
p*bdcB*	pBBR1-MCS-5-*npt*II-*bdcB* Gm^r	(Belhart *et al.*, 2023)
p*bdcA*	pBBR1-MCS-5-*npt*II- *bdcA* Gm^r	(Sisti *et al.*, 2013)

Table S2. Primers used and designed in this work.

Primer	Sequence	Purpose
BBpdeA_F1F	CCAAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATC AACGTCATTGCGATCG	Delete *bbpdeA*
BBpdeA_F1R	TCTAGAAAGTATAGGAACTTCGAAGCAGCTCCAGCCT ACACTGGCTGGACGGCGCGGCA	Delete *bbpdeA*
BBpdeA_F2F	AGGTCGACGGATCCCCGGAATTAATTCTCATGTTTGGG CAGATTCATGCGTATC	Delete *bbpdeA*
BBpdeA_F2R	AACAGCTATGACCATGATTACGAATTCGAGCTCGGTAC CATCGACCATCAGCGCG	Delete *bbpdeA*
mutpdeC-1F	CTGTTTTATCAGACCGCTTCTGCGTTCTGATCTGGACT TCTTGACGCCGAT	Delete *bbpdeC*
mutpdeC-1R	TTCGAGGATCAGATGCCACGTTATGCTGATCTTCCCGC CC	Delete *bbpdeC*
mutpdeC-2F	GGGCGGGAAGATCAGCATAACGTGGCATCTGATCCTC GAA	Delete *bbpdeC*
mutpdeC-2R	CGGATAACAATTTCACACAGGAAACAGCTATGCACCT GGGCATAGAAGTGCT	Delete *bbpdeC*
DegPF1F	CTGTTTTATCAGACCGCTTCTGCTTCTGATGCCACCAA CCTGCTGC	Delete *degP*
DegPF1R	TGCACCGCGACCCATCGCATCCACCGGCACA	Delete *degP*
DegPF2F	TGTGCCGGTGGATGCGATGGGTCGCGGTGCA	Delete *degP*
DegPF2R	CGGATAACAATTTCACACAGGAAACAGCTATGGAGGC GTCGACCACCA	Delete *degP*
mut_FHA_F1	CAGCTATGACCATGATTACGAATTCGCGTCAAAGGAAT GGCTGCG	Delete *fhaB*
mut_FHA_R1	GGCGCCGCCCGCGTTCACGGACAGCACCTGCCGCAC ACGCCAACATCAGG	Delete *fhaB*
mut_FHA_F2	TGGCCTGGGCCCTGATGTTGGCGTGTGCGGCAGGTGC TGTCCGTGAACGC	Delete *fhaB*
mut_FHA_R2	TAAAACGACGGCCAGTGCCAAGCTTTTCCTGCGGCAG CCACGGTC	Delete *fhaB*
mut_cyaA_F1	CAGCTATGACCATGATTACGAATTCCGAGCGTGTTGCG TGCGAGC	Delete *cyaA*
mut_cyaA_R1	GCTTCAGCGCCAGTTGACAGCCAGGGACTGCTGCAA GAACCAAACATCCA	Delete *cyaA*
mut_cyaA_F2	TACGACGTGCTGGATGTTTGGTTCTTGCAGCAGTCCC TGGCTGTCAACTG	Delete *cyaA*
mut_cyaA_R2	TAAAACGACGGCCAGTGCCAAGCTTCAGCGCCGGAA TGAACCAGC	Delete *cyaA*
Q30Verify F	GAGTCAGTGAGCGAGGAAG	pMQ30 sequencing
Q30Verify R	CAGACCGCTTCTGCGTTCTG	pMQ30 sequencing

Supplementary Figures

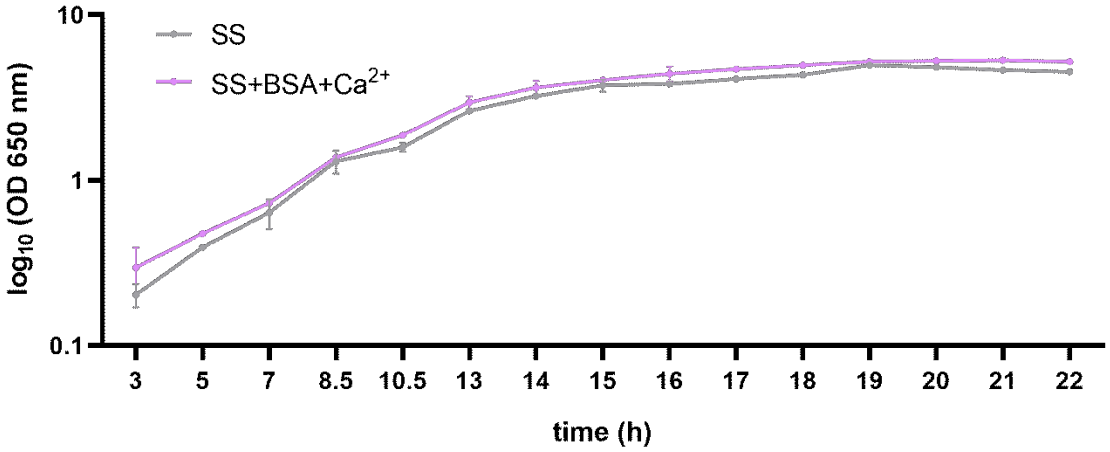

Figure S1. Growth kinetics of *B. bronchiseptica* wild type in SS medium or SS+BSA+Ca^{2+}. Cultures grown in BGA medium at 37°C were harvested and used to inoculate SS at initial OD_{650nm} = 0.1, followed by incubation at 37°C with shaking at 160 rpm. OD_{650nm} was recorded periodically. The results are the average of two independent experiments. There was no observable difference in growth between the two media.

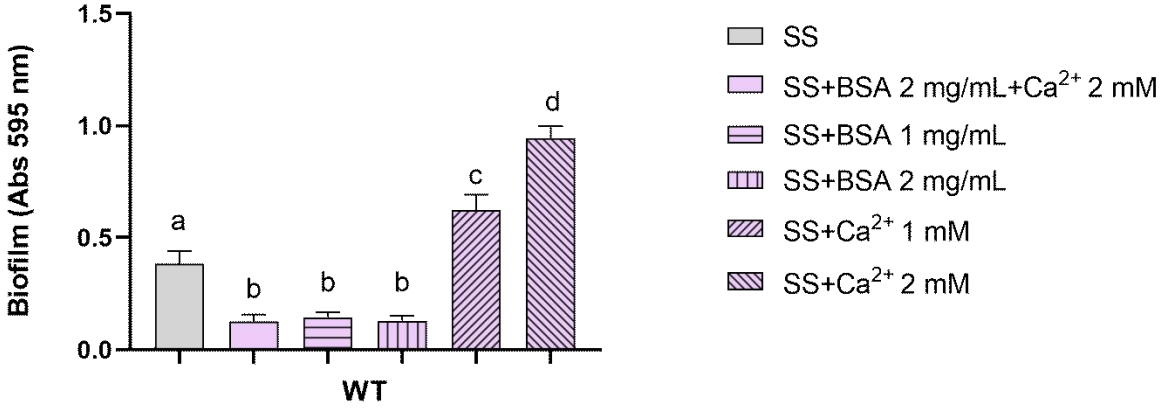

Figure S2. Effect of albumin and $CaCl_2$ separately and at different concentrations on biofilm formation. Biofilm formation on PVC 96-well of overnight cultures of wild type *B. bronchiseptica* 9.73 grown is SS or SS supplemented with BSA or $CaCl_2$. The biofilm formed was stained with CV and quantified after resuspension in 33% (v/v) acetic acid. Results are the average of at least three independent experiments. Different letters indicate significant differences ($p<0.01$; ANOVA).

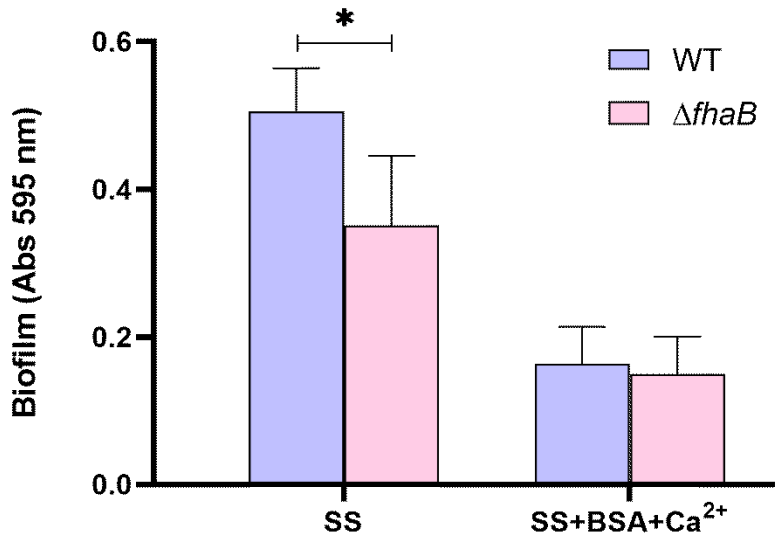

Figure S3. The Role of filamentous hemagglutinin in biofilm formation. Biofilm formation was assessed in PVC 96-well plates using overnight cultures of wild type *B. bronchiseptica*, and *Bb*Δ*fhaB* mutant grown in SS or SS supplemented with 2.0 mg/ml BSA and 2.0 mM $CaCl_2$. Biofilm was stained with CV and quantified after resuspension in 33% (v/v) acetic acid. Results are the average of at least three independent experiments. *Indicates a significant difference ($p<0.05$, ANOVA).

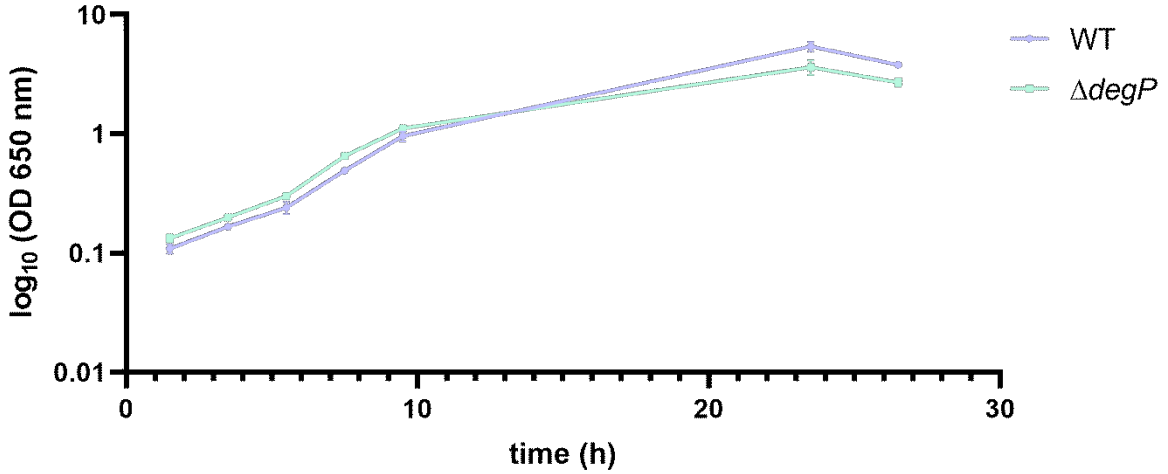

Figure S4. Growth kinetics of *B. bronchiseptica* wild type and Δ*degP* strains in SS. Cultures grown in BGA at 37°C were harvested and used to inoculate SS at initial DO_{650nm} = 0.1, followed by incubation at 37°C with shaking at 160 rpm. DO_{650nm} was recorded periodically. The results are average of two independent experiments. No significant differences were observed between the strains.

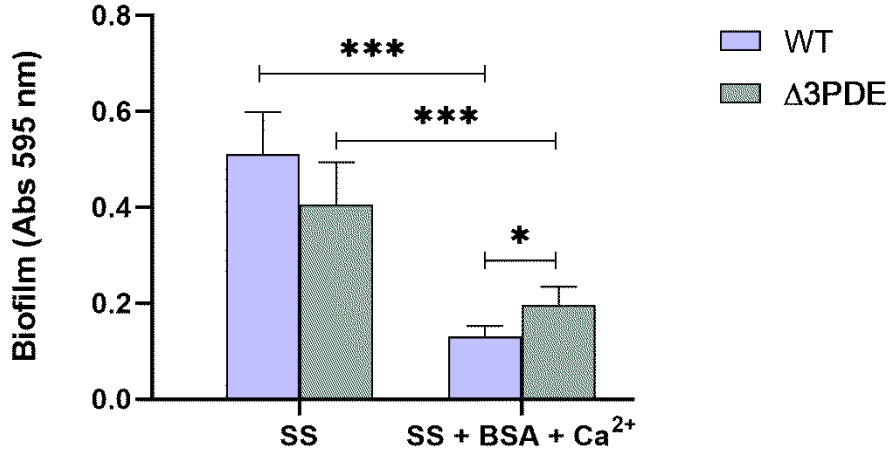

Figure S5. Influence of PDEs on biofilm formation. Biofilm formation on PVC 96-well plates of overnight cultures of wild type *B. bronchiseptica* and the Δ3PDE mutant grown in SS or SS supplemented with 2.0 mg/ml BSA and 2.0 mM $CaCl_2$. Biofilm was stained with CV and quantified after resuspension in 33% (v/v) acetic acid. Results are the average of at least three independent experiments. * or *** indicate significant differences ($p<0.05$ and $p<0.001$ respectively. ANOVA).

References.

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