

## **Fim3-dependent autoagglutination of *Bordetella pertussis***

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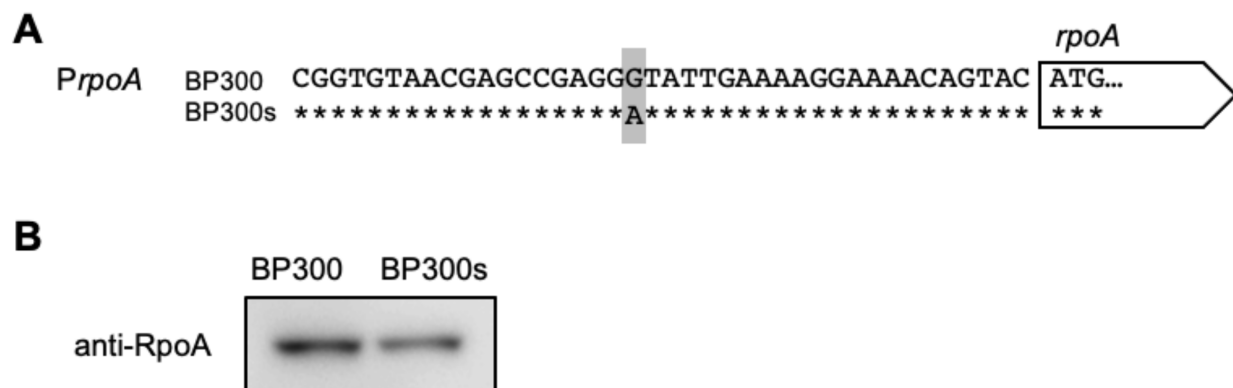
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**Table S1. Bacterial strains and plasmids for genetic engineering**

|         | Name                                                   | Characteristics                                                                                                                                                                                                               | Reference                                                                       |
|---------|--------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------|
| Strain  | <i>B. pertussis</i> Tohama I                           | Japanese clinical isolate in 1952, and widely used as laboratory strain. Agg <sup>-</sup> phenotype, MT87 carrying <i>ptxP1</i> , <i>ptxA2</i> , <i>prn1</i> , and <i>fim3A</i> alleles, Fim2 <sup>+</sup> /Fim3 <sup>-</sup> | Kamachi, K. et al. <i>Biologicals</i> 2009 Vol. 38 Pages 290-293                |
|         | <i>B. pertussis</i> BP300                              | Japanese clinical isolate in 2005, strong Agg <sup>+</sup> phenotype, MT26 carrying <i>ptxP3</i> , <i>ptxA1</i> , <i>prn2</i> and <i>fim3B</i> alleles, Fim2 <sup>-</sup> /Fim3 <sup>+</sup>                                  | This study                                                                      |
|         | <i>B. pertussis</i> BP300s                             | Agg <sup>-</sup> phenotype, derivative of BP300                                                                                                                                                                               | This study                                                                      |
|         | <i>B. pertussis</i> BP300Sm <sup>r</sup>               | Spontaneous streptomycin-resistant derivative of BP300, Sm <sup>r</sup>                                                                                                                                                       | This study                                                                      |
|         | <i>B. pertussis</i> BP300Sm <sup>r</sup> Δ <i>fim3</i> | <i>fim3</i> disrupted derivative of BP300Sm <sup>r</sup> , Sm <sup>r</sup>                                                                                                                                                    | This study                                                                      |
|         | <i>E. coli</i> DH5α                                    | Host strain used for general genetic methods                                                                                                                                                                                  | Nippon gene                                                                     |
|         | <i>E. coli</i> SM10λpir                                | thi thr leu tonA lacY supE recA::RP4-2-Tc::Mu Km λpir                                                                                                                                                                         | Simon R et al., <i>Nat Biotech.</i> 1983 Vol. 1 Issue 9 Pages 784-791           |
| Plasmid | pDONR221                                               | Cloning vector for Gateway system, Km <sup>r</sup> , <i>ccdB</i>                                                                                                                                                              | Invitrogen                                                                      |
|         | pABB-CRS2                                              | Low copy cloning vector for conjugation, Amp <sup>r</sup> , <i>sacB</i>                                                                                                                                                       | Sekiya K et al., <i>Proc Natl Acad Sci</i> 2001 Vol. 98 Issue 20 Pages 11638-43 |

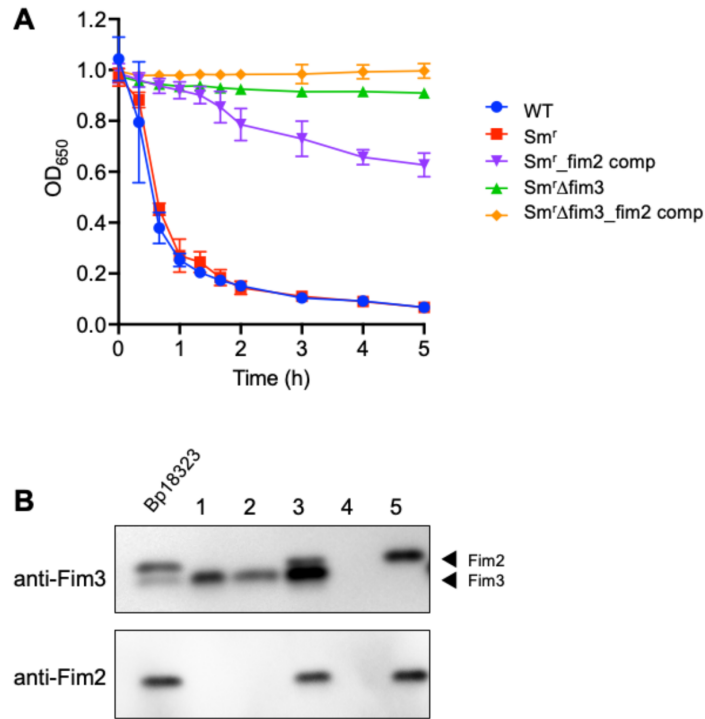
**Table S2. Primers, probes, and oligonucleotides used in this study**

| Application                       | Name          | Sequence (5' to 3')                            | Reference        |
|-----------------------------------|---------------|------------------------------------------------|------------------|
| DNA sequencing                    | M13 Primer M3 | GTAAAACGACGGCCAG                               | universal primer |
|                                   | M13 Primer RV | CAGGAAACAGCTATGAC                              | universal primer |
|                                   | 1567BF        | GAAGGCGGCGACTGCATGA                            | This study       |
|                                   | 1567CF        | CTCGTGCTGGATAGGCGCAG                           | This study       |
|                                   | 1567CF'       | CTGCGCCTATCCAGCACGAG                           | This study       |
|                                   | 1567R'        | CGGCCTGCAACAAGAACGAA                           | This study       |
|                                   | 1567R         | TTCGTTCTTGTTGCAGGCCG                           | This study       |
|                                   | fim3-F1       | ATGTCCAAGTTTTTCATACCC                          | This study       |
|                                   | fim3-innerR   | GGCTTGCGTGTTTTGTGTC                            | This study       |
|                                   | fim3-innerR'  | GACAAAACCACGCAAGCC                             | This study       |
|                                   | fim3-R        | TCACGGTGCTCGACGCCAA                            | This study       |
|                                   | fim3-R'       | TTGGCGTCGAGCACCGTGA                            | This study       |
|                                   | 1569AF        | GTCTTCCTTGCCGTGGGTC                            | This study       |
|                                   | 1569AF'       | GACCCACGGCAAGGAAGAC                            | This study       |
| $\Delta$ fim3 mutant construction | attB1-fim3F   | AAAAAGCAGGCTGGCGACTGCATGACGATCTC               | This study       |
|                                   | MP1-fim3      | GCTTGCGTGGTTTTGTCCGTCTACTAGGGCGAGGCGGCAAGGATAA | This study       |
|                                   | MP2-fim3      | TTATCCTTGCCGCCTCGCCCTAGTAGACGGACAAAACCACGCAAGC | This study       |
|                                   | attB2-fim3R   | AGAAAGCTGGGTTCCGGAACCGCGACCTACT                | This study       |
|                                   | attB1-adaptor | GGGGACAAGTTTGTACAAAAAAGCAGGCT                  | This study       |
|                                   | attB2-adaptor | GGGGACCACTTTGTACAAGAAAGCTGGGT                  | This study       |
| PCR/LDR for Pfim3 poly(C)         | Pfim3-F       | AGGCCATTTTCATTGCGCGA                           | This study       |
|                                   | Pfim3-R       | CTGATACTGAGGGGTGCCGT                           | This study       |
|                                   | fim3-11C      | ACAACCATCAGCCC                                 | This study       |
|                                   | fim3-12C      | AAACAACCATCAGCCCC                              | This study       |
|                                   | fim3-13C      | AAAAACAACCATCAGCCCCC                           | This study       |
|                                   | fim3-14C      | AAAAAAACAACCATCAGCCCCC                         | This study       |
|                                   | fim3-15C      | AAAAAAAAACAACCATCAGCCCCC                       | This study       |
|                                   | fim3-16C      | AAAAAAAAAAACAACCATCAGCCCCC                     | This study       |
|                                   | fim3-common   | Phos-CCCCCCCCGGACCTGATATTCTGATG-FAM            | This study       |

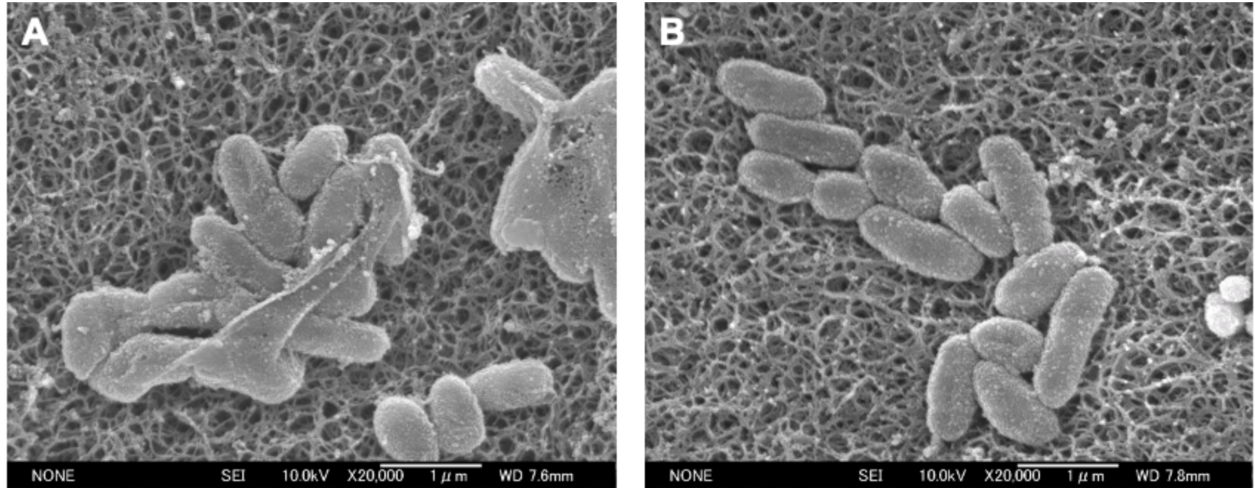


**Fig S1. Single-point mutation in promoter region of *rpoA* gene found in Agg<sup>-</sup> mutant, BP300s.**

(A) Upstream sequence of *rpoA* gene is shown, and the SNP position is gray-highlighted. (B) Immunoblot analysis with anti-RpoA antibody. Mouse anti-*E. coli* RNA polymerase  $\alpha$  (RpoA) was obtained from BioLegend (CA, USA).

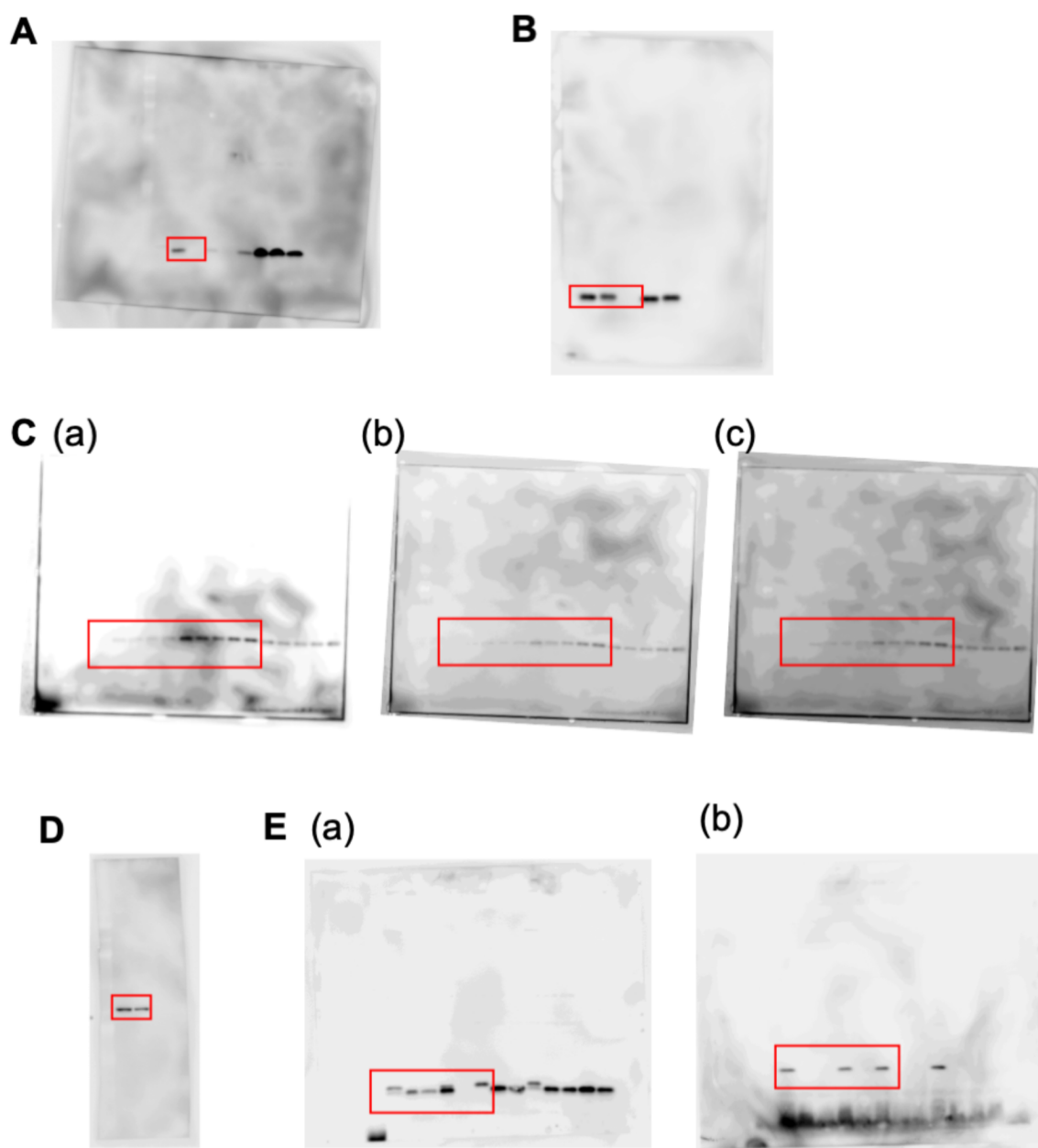


**Fig. S2. The role of Fim2 in *B. pertussis* autoagglutination.** (A) Autoagglutination assay of *B. pertussis* Δfim3 mutant and *fim2* gene-complemented mutants. (B) Immunoblot analysis using anti-Fim2 and anti-Fim3 antibodies. Rabbit anti-Fim2 immunoglobulin G (IgG) (number CSB-PA356626LA01BUA) and anti-Fim3 IgG (number CSB-PA322971LA01BUA) antibodies were purchased from Cusabio, China. *B. pertussis* BP300 (lane 1), BP300Sm<sup>r</sup> (lane 2), BP300Sm<sup>r</sup>/pRK-fim2 (lane 3), BP300Sm<sup>r</sup>Δfim3 (lane 4), and BP300Sm<sup>r</sup>Δfim3/pRK-fim2 (lane 5). Anti-Fim3 IgG reacts with both Fim2 and Fim3. Upper and lower arrows indicate Fim2 (22.5 kDa) and Fim3 (22.0 kDa), respectively. *B. pertussis* 18323 produces both Fim2 and Fim3 and is shown as a molecular standard.



**Fig. S3. SEM images of *B. pertussis* Agg<sup>+</sup> strain and Agg<sup>-</sup> mutant.**

(A) *B. pertussis* Agg<sup>+</sup> strain, BP300, (B) *B. pertussis* Agg<sup>-</sup> mutant, BP300s. For analysis of cell morphology, 10<sup>5</sup> cells/10  $\mu$ L of *B. pertussis* strains were spotted on Bordet–Gengou (BG) agar on the glass coverslips and incubated at 36 °C overnight. Scanning electron microscopy (SEM) was performed on a JEOL JSM-6700F microscope (Tokyo, Japan).



**Fig. S4. The original blots in this study.** (A) The original full-length blot of Figure 2D, (B) The original full-length blot of Figure 3A, (C) The blots shown in Figure 4; (a) the full-length blot in Figure 4, (b) and (C) are blots with different exposure or contrast, (D) The original full-length blot of Figure S1B, (E) The original full-length blots of Figure S4B; (a)

anti-Fim3 detection, (b) anti-Fim2 detection. The red-lined areas are clopped and shown in each figure.