Transcriptional and functional characterizations of multiple flagelin genes in spirochetes

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Table S1. Proteins identified in the purified PFs of *T. denticola* by using LC-MS/MS analysis.

| Accession | Description | Coverage (%) | Peptide(s) | Prec. (ppm) | Score (ppm) | Mascot score | Peptide ID | Entrez Gene | Gene Symbol | Gene Abundance | Abundance Fold | Spec Abundance |
|------------|-------------|--------------|------------|-------------|-------------|--------------|------------|-------------|-------------|---------------|---------------|---------------|
| T026686905.1 | flagellin (Propionibacterium denticola) | 82 | 16 | 1730 | 4 | 285 | 30.9 | 5.49 | 6822.74 | 16 | P06460.1 | 2481590 | ADA02071 | 10 | 1.01±1.2 |
| T026686843.1 | MSP (Propionibacterium denticola) | 78 | 27 | 887 | 27 | 549 | 58.2 | 6.83 | 3733.16 | 27 | P02070.1 | 2520351 | P159427 | 10 | 4.47±1.0 |
| T026686797.1 | flagellar filament outer layer protein FliA (Propionibacterium denticola) | 73 | 30 | 1359 | 12 | 349 | 39.3 | 5.33 | 4734.28 | 28 | P02060.2 | 2481594 | ADA02071 | 10 | 3.01±1.0 |
| T026686902.1 | flagellin (Propionibacterium denticola) | 67 | 28 | 975 | 4 | 280 | 31.3 | 6.13 | 3095.83 | 13 | P02060.1 | 2520352 | P159427 | 10 | 2.50±1.0 |
| T026686903.1 | flagellar hook protein Fig (Propionibacterium denticola) | 69 | 21 | 214 | 21 | 463 | 45.5 | 4.98 | 9213.55 | 21 | P02040.1 | 2481590 | P159427 | 10 | 6.79±1.0 |
| T026686642.1 | flagellin (Propionibacterium denticola) | 64 | 18 | 862 | 11 | 286 | 31.5 | 7.06 | 7966.83 | 15 | P06460.1 | 2481594 | ADA02071 | 10 | 6.13±1.0 |
| T026686461.1 | flagellar hook domain helix protein FliA (Propionibacterium denticola) | 55 | 12 | 459 | 12 | 234 | 26.2 | 5.54 | 2068.91 | 12 | P02040.1 | 2481595 | ADA02071 | 10 | 3.06±1.0 |
| T026686209.1 | flagellar hook-associated protein Fig (Propionibacterium denticola) | 48 | 19 | 67 | 19 | 623 | 69.5 | 4.94 | 245.73 | 19 | P06460.1 | 2481592 | ADA02071 | 10 | 0.48±1.0 |
| T026686646.1 | flagellar hook protein Fig (Propionibacterium denticola) | 48 | 12 | 43 | 12 | 567 | 64.8 | 5.89 | 115.44 | 12 | P02040.1 | 2481594 | ADA02071 | 10 | 2.44±1.0 |
| T026686466.1 | flagellar hook domain helix protein FliA (Propionibacterium denticola) | 44 | 9 | 23 | 9 | 264 | 28.8 | 5.73 | 86.94 | 9 | P02040.1 | 2481597 | ADA02071 | 10 | 3.06±1.0 |
| T026686608.1 | flagellar basal body-associated FliA family protein (Propionibacterium denticola) | 36 | 8 | 28 | 8 | 264 | 28.8 | 5.73 | 86.94 | 9 | P02040.1 | 2481597 | ADA02071 | 10 | 3.06±1.0 |
| T026686781.1 | flagellar flagella basal body-associated Fig (Propionibacterium denticola) | 34 | 8 | 16 | 6 | 270 | 30.2 | 4.94 | 41.94 | 6 | P02040.1 | 2481598 | ADA02071 | 10 | 0.99±1.0 |
| T026686781.2 | flagellar flagella basal body-associated Fig (Propionibacterium denticola) | 34 | 8 | 16 | 6 | 270 | 30.2 | 4.94 | 41.94 | 6 | P02040.1 | 2481598 | ADA02071 | 10 | 0.99±1.0 |
| T026686640.1 | flagellar flagella basal body-associated Fig (Propionibacterium denticola) | 33 | 3 | 3 | 3 | 159 | 15.8 | 7.5 | 11.35 | 3 | P02040.1 | 2481596 | ADA02071 | 10 | 0.15±1.0 |

Table S2. Characterization of *T. denticola* wild type and four flagellar filament gene deletion mutants by using cryo-electron tomography.

|         | Cell length (μm) | PFs length (μm) | PFs length (old pole, μm) | PFs diameter (nm) |
|---------|------------------|------------------|-----------------------------|-------------------|
| **WT**  |                  |                  |                             |                   |
| Cell 1  | 4.4              | 6.5              | 19.5 ± 1.7                  | 14.2 ± 1.4        |
| Cell 2  | 3.0              | 5.1              | 13.9 ± 1.5                  |                   |
| **ΔflaA** |                 |                  |                             |                   |
| Cell 1  | 3.0              | 8.0              | 19.9 ± 2.0                  | 13.0 ± 1.1        |
| Cell 2  | 3.6              | 12.0             | 21.1 ± 1.4                  | 13.6 ± 1.6        |
| **ΔflaB1** |                |                  |                             |                   |
| Cell 1  | 1.2              | 1.5              |                             |                   |
| Cell 2  | 3.0              | 4.3              |                             |                   |
| **ΔflaB2** |                |                  |                             |                   |
| Cell 1  | 2.4              | 4.4              | 20.6 ± 2.0                  | 13.4 ± 1.6        |
| Cell 2  | 3.0              | 5.0              |                             |                   |
| **ΔflaB3** |                |                  |                             |                   |
| Cell 1  | 2.0              | 3.0              |                             |                   |

Diagram: Depiction of the amplification primers (P11, P12, P13, P14) associated with the TDE002, TDE004 (ΔflaB2), and TDE006 regions. Genes of interest are indicated with their respective sizes (500 bp, 200 bp).
**Figure S1.** Co-RT-PCR analysis of TDE1002-TDE1006 genes. This experiment was performed as previously documented (Kurniyati et al., 2019). Two pairs of primers that bridge flaB2 and its flanking genes were designed and used for co-RT-PCR. For each co-RT-PCR reaction, a parallel PCR reaction was performed and used as a positive control. The resultant co-RT-PCR and PCR products were detected in 2% agarose gel electrophoresis.

**Figure S2.** Representative cryo-electron microscopic images of PFs isolated from ΔflaB1, ΔflaB2, and ΔflaB3 mutants.

**Figure S3.** Representative TEM images of PFs isolated from T. denticola wild type (WT) and four flagellar filament gene deletion mutants.

**Figure S4.** Characterizations of two flaB2 gene replaced mutants: ΔflaB2/B1 and ΔflaB2/B3. (A) SDS-PAGE analysis of PFs isolated from WT, ΔflaB2, ΔflaB2/B1, and ΔflaB2/B3 mutants. (B) Western-blot analysis of isolated PFs. For the immunoblotting, antibodies against T. denticola DnaK (αDnaK), FlaA (αFlaA), and T. pallidum FlaB (αFlaB) were used.
Figure S5. Characterizations of six double deletion mutants of *T. denticola*. (A) Whole cell lysate immunoblotting analysis of WT and six flagellar filament gene double deletion mutants. The blots were probed with antibodies against *T. denticola* DnaK (αDnaK), FlaA (αFlaA), and *T. pallidum* FlaB (αFlaB), respectively. DnaK was used as a loading control. (B) 2D gel electrophoresis of six double mutants, followed by immunoblotting with antibodies against *T. denticola* FlaA (αFlaA) and *T. pallidum* FlaB (αFlaB). (C) Swimming plate assay. This assay was carried out on 0.35% agarose plates containing the TYGVS medium diluted 1:1 with PBS. The plates were incubated anaerobically at 37°C for 3 days to allow the cells to swim out. Δtap1, a previously constructed non-motile mutant (Limberger et al., 1999), was used as a control to determine the initial inoculum sizes. The sizes of swimming rings from five different plates were measured and averaged. (D) Cell tracking analysis. *T. denticola* cells were tracked in the presence of 1% methylcellulose, as previously described. The results are expressed as the mean of μm/s ± standard errors of mean (SEM). WT: wild type; ΔAB1: ΔflaAflaB1; ΔAB2: ΔflaAflaB2; ΔAB3: ΔflaAflaB3; ΔB1B2: ΔflaB1flaB2; ΔB1B3: ΔflaB1flaB3; and ΔB2B3: ΔflaB2flaB3.