The anti-sigma[28] factor FlgM regulates the flagellin gene expression and flagellar polarity of *Treponema denticola*

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Table S1. Oligonucleotide primers used in this study

Primers	Sequences (5'-3')	Note[a]
P_1	CACCATGATAGAAAAATTGGGCGG	TDE0201 recombinant protein; [F]
P_2	TTAAAATCCCAAAGAGTC	TDE0201 recombinant protein; [R]
P_3	CAAACGGGACATTGACGCTGC	5` portion for TDE0201 inactivation; [F]
P_4	<u>TCTAGA</u>GTTTTCCTGCCTTCCTAACC	5` portion for TDE0201 inactivation; [R]
P_5	<u>TCTAGA</u>CGATAGCTTCCGCTATTGC	Erythromycin cassette (*ERM-F/AM*); [F]
P_6	<u>TCTAGA</u>TTTATCTACATTCCCTTTAGTAACG	Erythromycin cassette (*ERM-F/AM*); [R]
P_7	<u>TCTAGA</u>TTAAAATAATTTATTGATAAAAG	3` portion for TDE0201 inactivation; [F]
P_8	GTTTCCGTCAGCTTTGTGGG	3` portion for TDE0201 inactivation; [R]
P_9	CGTTGCTGCTGCGTAACATCTATTAAAATCCCAAAGAGTCTAA	5` portion for TDE0201 complementation; [R]
P_{10}	ATGTTACGCAGCAGCAACG	Gentamicin cassette (*aacC1*); [F]
P_{11}	TTAGGTGGCGGTACTTGGGTC	Gentamicin cassette (*aacC1*); [R]
P_{12}	GACCCAAGTACCGCCACCTAAGTATTTTGGATCAAACAGCAG	3` portion for TDE0201 complementation; [F]
P_{13}	<u>GGATCC</u>ATGGCAAATACGGATTATG	5` portion for FliA-FlgM expression; [F]
P_{14}	CCCAATTTTTCTATCATCATCTATTTTATGCCCTTTGTAAC	5` portion for FliA-FlgM expression; [R]
P_{15}	GTTACAAAGGGCATAAAATAGATGATGATAGAAAAATTGGG	3` portion for FliA-FlgM expression; [F]
P_{16}	<u>CTGCAG</u>CTACTTGTCGTCATCGTCCTTGTAGTCAAATCCCAAAGAGTCTAAAAT	3` portion for FliA-FlgM expression; [R]
P_{17}	CTAACTTTTTTCTTAAGTCTTTTTTTAGTTCTCCGATAAATGAACTAAGCAC	*flaB2* promoter DNA probe for EMSA; [F]

P$_{18}$	GTGCTTAGTTCATTTATCGGAGAACTA AAAAAAGACTTAAGAAAAAGTTAG	*flaB2* promoter DNA probe for EMSA; [R]
P$_{19}$	CCGTCCATTGTAGGCTTAC	qRT-PCR, *dnaK*; [F]
P$_{20}$	CCGTCAATGTCGATTCGTAC	qRT-PCR, *dnaK*; [R]
P$_{21}$	ATGAAAAAAACATTTATACTTG	qRT-PCR, *flaA*; [F]
P$_{22}$	CGAACAGAAAGAGGATTCTG	qRT-PCR, *flaA*; [R]
P$_{23}$	ATGATTATCAATCACAACATG	qRT-PCR, *flaB1*; [F]
P$_{24}$	GAAGCCTGAACAGCGAGTTC	qRT-PCR, *flaB1*; [R]
P$_{25}$	CACAGAGGACATTGGGACAC	qRT-PCR, *flaB2*; [F]
P$_{26}$	CTGAGTTCGCGGATTCTCTG	qRT-PCR, *flaB2*; [R]
P$_{27}$	GCACAGAGACAGGGAGGAGTC	qRT-PCR, *flaB3*; [F]
P$_{28}$	CCGTTTGAGGCCTGTATAGC	qRT-PCR, *flaB3*; [R]
P$_{29}$	ATGGCAAATACGGATTATG	qRT-PCR, *TDE2683*; [F]
P$_{30}$	<u>AGATCT</u>CAGCTCATCAAAGATAGCTC	qRT-PCR, *TDE2683*; [R]
P$_{31}$	GCGCACAATAGGATTGGGTG	qRT-PCR, *TDE1346*, σ70; [F]
P$_{32}$	CTTCTTCGTATGTATCATCC	qRT-PCR, *TDE1346*, σ70; [R]
P$_{33}$	GCTTGCATGTTTTGCAGATGC	Co-RT-PCR, *TDE0199*; [R]
P$_{34}$	GCTTCATTTACAGCCCTGCACG	Co-RT-PCR, *TDE0200*; [R]
P$_{35}$	GCCGAAATAGATAGTAACCG	Co-RT-PCR, *TDE0200*; [F]

P$_{36}$	GAGCTTCAGGAGATACTTGC	Co-RT-PCR, *TDE0201*; [R]
P$_{37}$	GATAGAAGAAGTTGCAAGAA	Co-RT-PCR, *TDE0201*; [F]
P$_{38}$	GCGGACGGATTCGGTTCTCC	Co-RT-PCR, *TDE0202*; [F]
P$_{39}$	GATTACGCCAAGCTTCGGAGGCGGCGG CTTCATTTACAGCCCTGC	5' RACE for TDE0201; [R]
P$_{40}$	<u>AAGCTT</u>ATAGAAAAATTGGGCGGAATT G	TDE0201 protein expression; [F]
P$_{41}$	<u>GAATTC</u>AAATCCCAAAGAGTCTAAAAT	TDE0201 protein expression; [R]
P$_{42}$	<u>CTGCAG</u>CTACTTGTCGTCATCGTCCTTG TAGTCAATACTGTCTATATGAAGGA TC	3`portion for FliA-FlgM over-expression; [R]
P$_{43}$	<u>GGATCC</u>GTGAATTCACTCTATACCGC	5`portion for *E. coli* FliA-FlgM overexpression; [F]
P$_{44}$	CCCAATTTTTCTATCATCATTTATAACT TACCCAGTTTAGTGC	5`portion for *E. coli* FliA-FlgM overexpression; [R]
P$_{45}$	GCACTAAACTGGGTAAGTTATAAATGA TGATAGAAAAATTGGG	3`portion for *E. coli* FliA-FlgM overexpression; [F]

[a] Underlined sequences are engineered restriction cut sites for DNA cloning; [F] forward; [R] reverse.
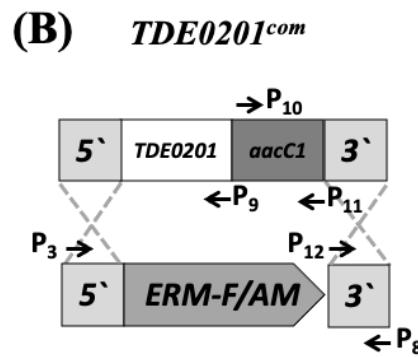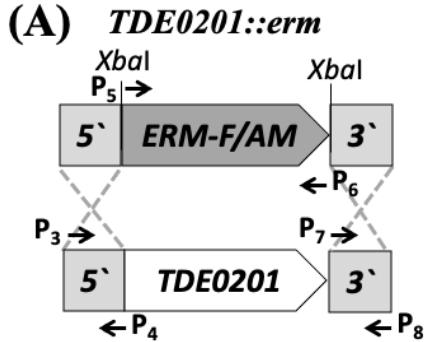
Figure S1. Diagrams showing construction of *TDE0201::erm* and *TDE0201^{com}*. **(A)** *TDE0201::erm* was constructed to *in-frame* replace *TDE0201* with the *ERM-F/AM* cassette. **(B)** *TDE0201^{com}* was constructed to replace the *ERM-F/AM* cassette in the *ΔflgM* mutant with *TDE0201-aacC1*. These constructs were constructed by two-step PCR. Arrows represent the relative positions and orientations of these primers, which are listed in Table S1. *ERM-F/AM* is an erythromycin-resistance cassette; *aacC1* is a gentamycin-resistance cassette.
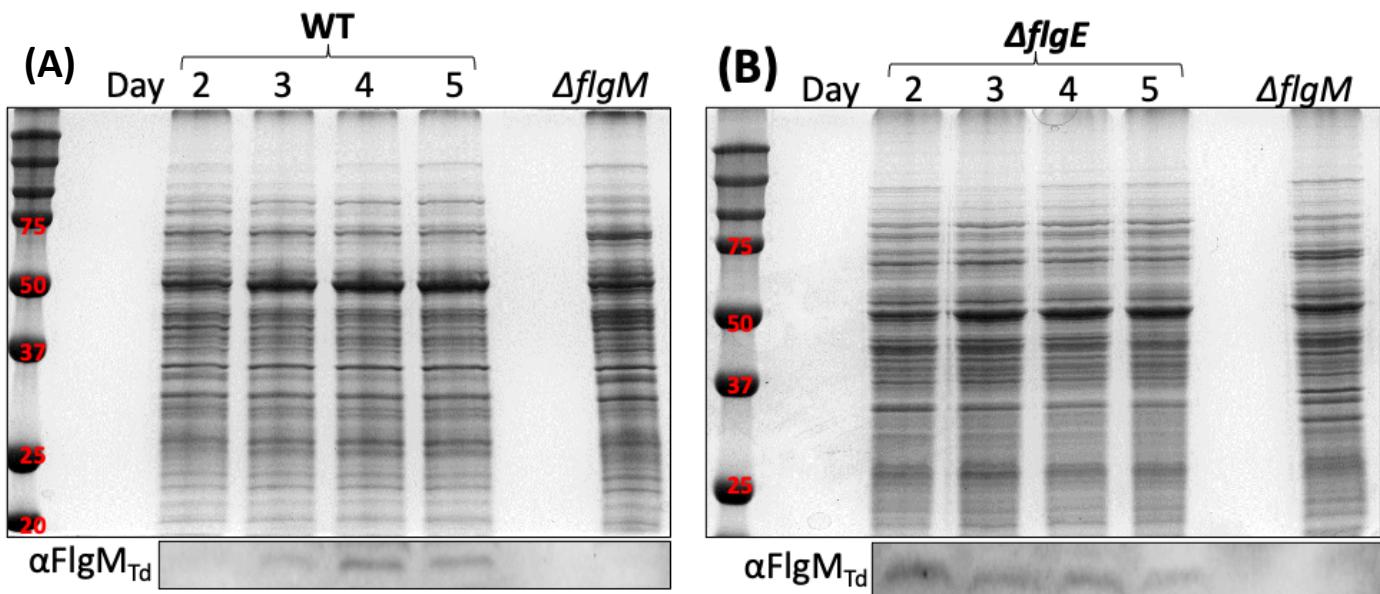
Figure S2. Measuring the level of FlgM$_{Td}$ throughout the growth phase of *T. denticola*. For this study, wild type (A), and a previously constructed *flgE*-deficient mutant (*ΔflgE*) of *T. denticola* (B) were used. A total of 2×10^6 cells/ml of late-log phase *T. denticola* cultures was inoculated into the TYGVS medium. *T. denticola* cells in the cultures were enumerated every 24 hours using a Petroff Hausser counting chamber (Hausser Scientific, Horsham, PA). Samples were harvested at different time points and subjected to immunoblotting analysis using the FlgM$_{Td}$ antibody (αFlgM$_{Td}$).