

**A surface-exposed neuraminidase affects complement resistance and virulence of the oral  
spirochete *Treponema denticola***

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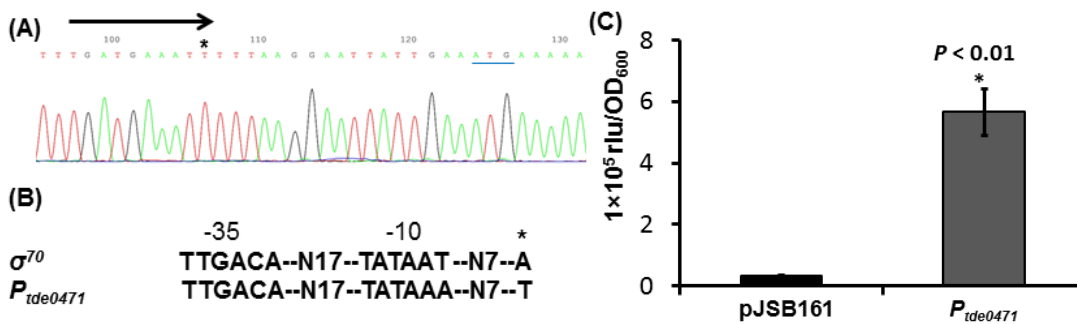
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**Table S1.** Oligonucleotide primers used in this study

Primers	Sequences (5'-3')	Note <sup>a</sup>
P <sub>1</sub>	CACCATGAAAAATAGCATATCAG	TDE0471 recombinant protein; [F]
P <sub>2</sub>	CTGCAGTTCTTTCCAGTGCAGTTTTTC	TDE0471 recombinant protein; [R]
P <sub>3</sub>	<u>GGATCC</u> GTTTTGGGAGATGCGTCTAC	TDE0471 truncated protein; [F]
P <sub>4</sub>	<u>AAGCTT</u> TTTCTTTCCAGTGCAGTTTTTC	TDE0471 truncated protein; [R]
P <sub>5</sub>	<u>TCTAGAC</u> GATAGCTTCCGCTATTGCT	Erythromycin cassette ( <i>erm<sup>R</sup></i> ); [F]
P <sub>6</sub>	<u>TCTAGAT</u> TTTATCTACATTCCCTTTAGT AACG	Erythromycin cassette ( <i>erm<sup>R</sup></i> ); [R]
P <sub>7</sub>	CTCTCATTC CGCCTTTTACGC	<i>TDE0471</i> inactivation; [F]
P <sub>8</sub>	GATGCGATAATCGCTGAGGAG	<i>TDE0471</i> inactivation; [R]
P <sub>9</sub>	<u>AGATCT</u> CGATAGCTTCCGCTATTGCT TTTTTG	5'-portion of <i>erm<sup>R</sup></i> for complementation; [F]
P <sub>10</sub>	GCTGTTTTAAGGAGAAGTATAATACA CCCGACTTTGAACTACGAAG	5'-portion of <i>erm<sup>R</sup></i> for complementation; [R]
P <sub>11</sub>	CTTCGTAGTTCAAAGTCGGGTGTATT ATACTTCTCCTTAAAACAGC	<i>aacCI</i> for complementation; [F]
P <sub>12</sub>	<u>TCTAGAT</u> TAGGTGGCGGTACTTGGGT	<i>aacCI</i> for complementation; [R]
P <sub>13</sub>	<u>TCTAGAT</u> ATCCTCCAATAATCCTATC	<i>TDE0471</i> for complementation; [F]

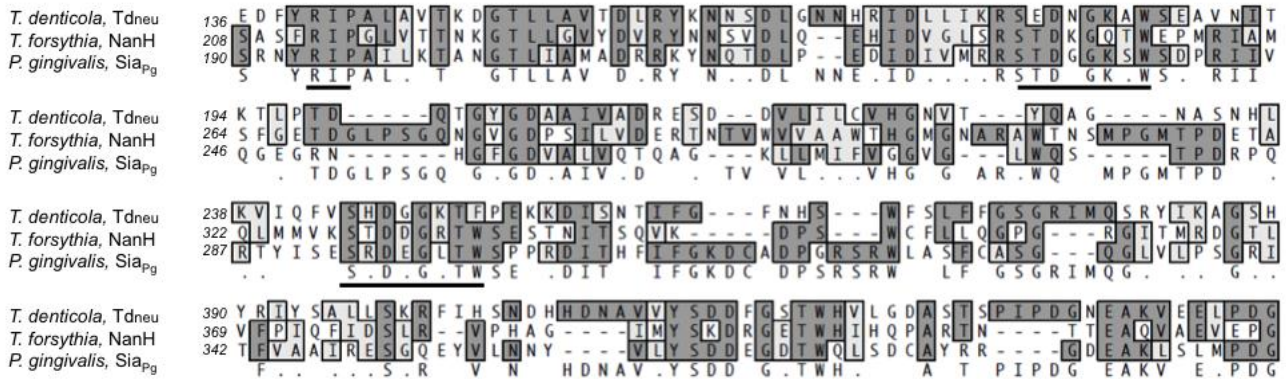
P <sub>14</sub>	CAAAAAAATCATCTTGACAACCTTATT CTTTCCAGTGCAGTT	<i>TDE0471</i> for complementation; [R]
P <sub>15</sub>	AACTGCACTGGAAAGAATAAGTTGT CAAGATGATTTTTTTTG	3'-portion of <i>erm</i> <sup>R</sup> for complementation; [F]
P <sub>16</sub>	<u>AGATCT</u> TTTATCTACATTCCTTTAGT AACG	3'-portion of <i>erm</i> <sup>R</sup> for complementation; [R]
P <sub>17</sub>	CCGGCTTGAAGAAGATTGGC	Flanking region of <i>TDE0471</i> , mutant PCR analysis; [F]
P <sub>18</sub>	TTACGTTTCCGCTCCATCGC	<i>erm</i> <sup>R</sup> , mutant PCR analysis; [R]
P <sub>19</sub>	CAGAGTGAGAGAAAGGGGGA	<i>erm</i> <sup>R</sup> , mutant PCR analysis; [F]
P <sub>20</sub>	<u>AGATCT</u> TATATACCTCCAATAATCC	<i>TDE0471</i> promoter; [F]
P <sub>21</sub>	<u>CATATG</u> TTCAATTCCTTAAAAGC	<i>TDE0471</i> promoter; [R]

<sup>a</sup> Underlined sequences are engineered restriction cut sites for DNA cloning; [F] forward; [R] reverse.

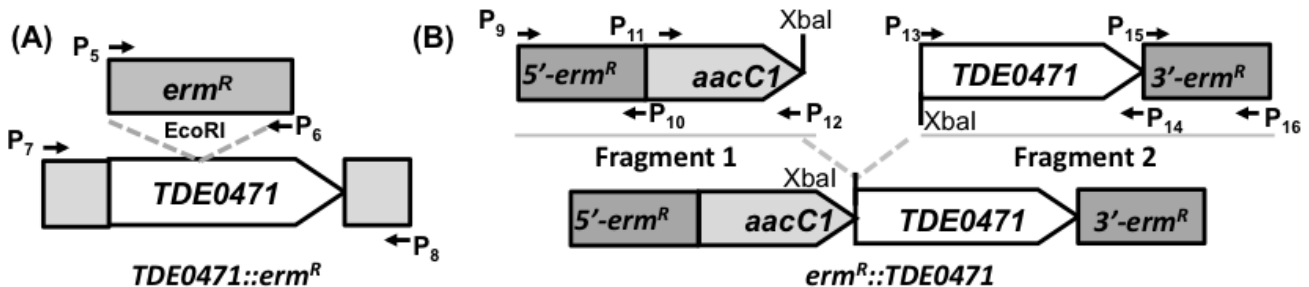


**Fig.S1.** The *TDE0471* gene is regulated by a sigma<sup>70</sup> promoter. (A) Identifying the transcriptional start site of *TDE0471* by 5'-RLM-RACE analysis. Arrow show the sequencing direction, and \*point to the identified transcriptional start site. (B) Sequence comparison between the *E. coli* sigma<sup>70</sup> promoter (Typas *et al.*, 2007) and the consensus sequence upstream of *TDE0471* (designated as *P<sub>tde0471</sub>*). (C)

Transcriptional analysis of *P<sub>ide0471</sub>* using the luciferase gene as a reporter. For this assay, *P<sub>ide0471</sub>* was fused to the luciferase gene within the plasmid pJSB161 and the luciferase activity was measured using a commercial luciferase assay kit and a Veritas Microplate Luminometer as previously described (Sze and Li, 2011). The promoterless pJSB161 plasmid was used as a negative control. The data was expressed as relative luciferase units per 10<sup>5</sup> cells (RLU/10<sup>5</sup> cells).

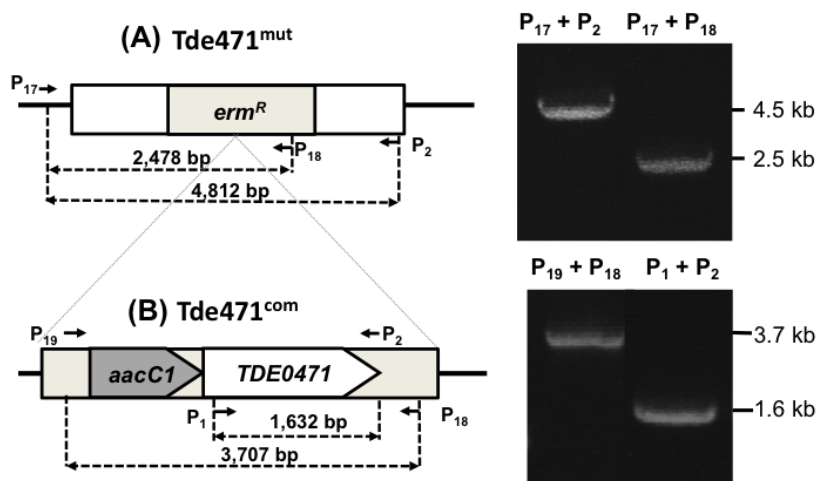


**Fig.S2.** Sequence comparison of neuraminidases from ‘red-complex’ bacteria. The underlined sequences represent the conserved domains identified in neuraminidases, including a RIP motif and three “Asp-box” motifs (Ser/Thr-X-Asp-[X]-Gly-X-Thr-Trp/Phe). Only one part of aligned sequences is presented. The aligned proteins include: *T. denticola* (TDE0471; NP\_971085), *P. gingivalis* (PG0352; NP\_904664), and *T. forsythia* (TF0035; YP\_005015051). The alignments were conducted using the program MacVector 10.6.



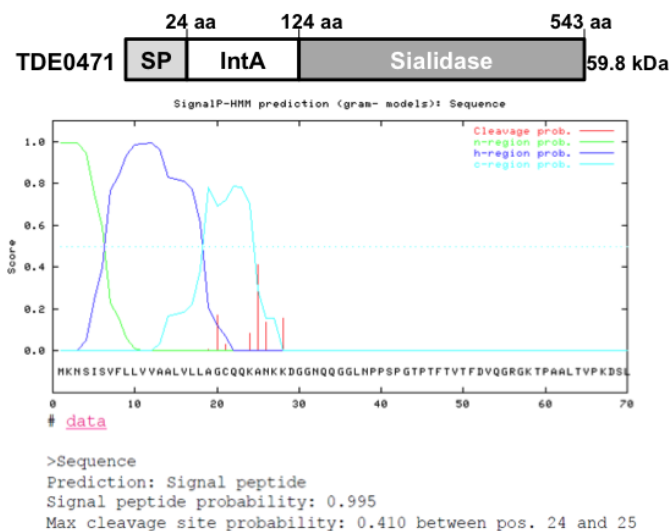
**Fig.S3.** Diagrams showing construction of the *TDE0471::erm<sup>R</sup>* vector (A) for the targeted mutagenesis of *TDE0471*, and the *erm<sup>R</sup>::TDE0471* vector (B) for *cis*-complementing the *TDE0471* isogenic mutant.

*Erm<sup>R</sup>::TDE0471* was used to complement the *TDE0471* mutant by inserting the *aacC1-TDE0471* construct into the *erm<sup>R</sup>* cassette on the chromosome of the mutant. *Erm<sup>R</sup>::TDE0471* was constructed by two step PCR and DNA cloning. The 5'-portion of *erm<sup>R</sup>* and a previously constructed gentamicin resistance cassette (*aacC1*) (Bian *et al.*, 2012) were PCR amplified with primers P<sub>9</sub>/P<sub>10</sub> and P<sub>11</sub>/P<sub>12</sub>, respectively, and then fused together with primers P<sub>9</sub>/P<sub>12</sub>, generating Fragment 1. The 3'-portion of *erm<sup>R</sup>* and a DNA fragment containing the full length of *TDE0471* and its upstream promoter sequence were PCR amplified with primers P<sub>15</sub>/P<sub>16</sub> and P<sub>13</sub>/P<sub>14</sub>, respectively, and then fused together by PCR using primers P<sub>13</sub>/P<sub>16</sub>, generating Fragment 2. The two obtained DNA fragments were cloned into the pGEM-T easy vector and then fused together at an engineered XbaI cleavage site. Arrows represent the approximate positions and orientations of the primers used for PCR amplifications. The primer sequences are listed in Table S1.

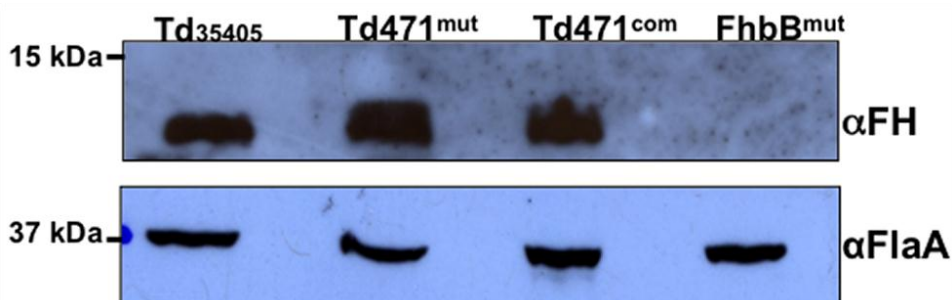


**Fig.S4.** Characterizations of the *Tde471<sup>mut</sup>* mutant (A) and its cognate complemented strain *Tde471<sup>com</sup>* (B) by PCR analysis. The diagrams illustrating how the PCR analysis is designed; the figures on the right are the PCR results. Arrows represent the relative positions and orientations of these primers; the numbers are predicted sizes of PCR products generated by the corresponding primers. The primer P<sub>17</sub> is located at the flanking region of *TDE0471*, P<sub>18</sub> at the 3' end of *erm<sup>R</sup>*, P<sub>2</sub> at the 3' end of *TDE0471*, and P<sub>19</sub>

at the 5' end of *erm<sup>R</sup>*. The sequences of these primers are listed in Table S1. Two pairs of primers were used for each strain. The numbers are approximate sizes of detected PCR products generated by the corresponding primers as labeled.



**Fig.S5.** A diagram (the top) illustrating the domain composition of TDE0471; the bottom is the prediction of signal peptide using the SignalP 4.0 server (<http://www.cbs.dtu.dk/services/SignalP/>).



**Fig.S6.** Detection of factor H (FH) binding affinity to Td35405, Tde471<sup>mut</sup>, Tde471<sup>com</sup>, and FhbB<sup>mut</sup> strains. The affinity ligand binding immunoblot (ALBI) assay was performed as previously described (McDowell *et al.*, 2005). For this assay, equal amounts of Td35405, Tde471<sup>mut</sup>, Tde471<sup>com</sup>, and FhbB<sup>mut</sup> whole-cell lysates were analyzed by SDS-PAGE and transferred to PVDF membranes. The obtained blots were probed with FH, followed by a monoclonal antibody against FH.

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