



**Figure S1. Motility behavior of the complemented  $\Delta$ MC strain in the presence of mucin.** 2D traces of complemented  $\Delta$ MC cells were captured by dark-field microscopy of bacterial motility in M9 media without or with 2% mucin. Different single-cell trajectories in x and y are indicated by different colors. Open circles denote the start position for each trace and green circles indicate flicks. Traces from a single representative experiment are shown.

**Table S1. Primers used in this study**

Primer	Purpose	Sequence
<i>EclacZ</i> -F	Amplify <i>E. coli lacZ</i>	ATGACCATGATTACGGATTAC
<i>EclacZ</i> -R		GCCGGCGCCGCTCTAGTTATTTTTGACACCAGACCAAC
Vch $\Delta P_{lacZ}$ -1	Delete promoter of chromosomal <i>V. cholerae lacZ</i>	GTACCGGGTTGAGAAGCGGTGTAAGTGAAGTGCATCATC TCTGGTGTCAGCAC
Vch $\Delta P_{lacZ}$ -2		AAATAGAGGTCGATATTGACC
Vch $\Delta P_{lacZ}$ -3		GGTCAATATCGACCTCTATTCGCAACTTCTCCGATATTCT TC
Vch $\Delta P_{lacZ}$ -4		TTGCTACGCCTGAATAAGTGATAGGGCCCGATCCCCTGA GTGATTTCTACTTTGCG
Vch $\Delta vipA$ -1	Construct the <i>V. cholerae</i> $\Delta MC$ mutant	CGCTTATGAGCTCGCGCTG
Vch $\Delta vipA$ -TpK7-2		GTCGACGGATCCCCGGAATCATATTACGTCTCCAATACCT AT
Vch $\Delta hsiF$ -TpK7-3		GAAGCAGCTCCAGCCTACATAACCTCTATGACGCAAGAC AA
Vch $\Delta hsiF$ -4		GTATCTTGCCAACCAACCACT
Vch <i>vipA-hsiF</i> -F	Amplify <i>vipA-hsiF</i> region with native promoter for cloning into pSW23T- <i>lacZ</i> <sup>Vc400</sup>	GCCATGTATCAGTGACGAAACAACAACACTCTGTGACA TGCC
Vch <i>vipA-hsiF</i> -R		GACGCGATGGGTCGCGCTGTTAAAACACTCGATATTTTCT G
Vch <i>P<sub>vipA</sub></i> -F	Amplify the <i>V. cholerae vipA</i> promoter for <i>lacZ</i> reporter construction	GACTCACTATAGGGCGAATTGCAACAACACTCTGTGACAT GGC
Vch <i>P<sub>vipA</sub></i> -R		GTGAATCCGTAATTTTGGGAGCTACACTTCCTTC
TmR-F	Amplify the Tm resistance cassette	ATCCGGGGATCCGTCGAC
TmR-R		TGTAGGCTGGAGCTGCTTC
Vch $\Delta cheY3$ -1	Construct the <i>V. cholerae</i> $\Delta cheY3$ mutant	ATTAACATTAGTGACAGAACGG
Vch $\Delta cheY3$ -TpK7-2		GTCGACGGATCCCCGGAATTGCCTCCACTGAGTTTGAGAT C
Vch $\Delta cheY3$ -TpK73		GAAGCAGCTCCAGCCTACATTATAAACGCTCAAAAATTTT GTC
Vch $\Delta cheY3$ -4		CCATTTTCGAAAATGTGGAACAC
Vch <i>cheY3</i> -F	Amplify <i>cheY3</i> fused to <i>P<sub>tac</sub></i> for cloning into pSW23T- <i>lacZ</i> <sup>Vc400</sup>	GACTCACTATAGGGCGAATTGGAGGCAATTTTGAATAAA AACATG
Vch <i>cheY3</i> -R		GCCGGCGCCGCTCTAGTTATAAACGCTCAAAAATTTTGT C

**Table S2. Plasmid used in this study**

Plasmid	Purpose	Source
pNKTXI-Scel	mini <i>Tn10</i> transposon mutagenesis plasmid	(3)
pMMB-TfoX	Allelic replacement of target <i>V. cholerae</i> genes by MuGENT	(4)
pRE112	Conditionally replicating plasmid for generating markerless deletions; <i>cat</i> , <i>sacB1</i> , <i>RP4oriT</i> , <i>R6Kgori pir</i>	(1)
pRE112- $\Delta P_{lacZ}^{Vch}$	Markerless deletion of the chromosomal <i>lacZ</i> <sup>Vch</sup> promoter	This work
pSW23T	Conditionally replicating plasmid; <i>cat</i> , <i>RP4oriT</i> , <i>R6Kgori pir</i>	(2)
pSW23T- <i>lacZ</i> <sup>Vc400</sup>	Integration into the chromosomal <i>lacZ</i>	This work
pSW23T- <i>lacZ</i> <sup>Vc400</sup> - <i>P<sub>vipA</sub>lacZ<sup>Ec</sup></i>	Reporter plasmid for <i>P<sub>vipA</sub></i> expression	This work
pSW23T- <i>lacZ</i> <sup>Vc400</sup> - <i>vipA-hsiF</i>	Complementation of $\Delta$ MC	This work

## References

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