Supporting Information

The putative oligosaccharide translocase SypK connects biofilm formation with quorum signaling in *Vibrio fischeri*

Tim Miyashiro[1,2,*], Dane Oehlert[2], Valerie A. Ray[3], Karen L. Visick[3], and Edward G. Ruby[2]

[1] Department of Biochemistry and Molecular Biology
Eberly College of Science
The Pennsylvania State University
University Park, PA 16802, USA

[2] Department of Medical Microbiology and Immunology
University of Wisconsin School of Medicine and Public Health
Madison, WI 53706, USA

[3] Department of Microbiology and Immunology
Loyola University Medical Center
Maywood, IL 60153, USA

* Corresponding author
Email: tim14@psu.edu
Phone: (+1) 814 865 1916
Fax: (+1) 814 863 7024

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Strain Construction

Natural transformation to generate DRO222. Template DNA for natural transformation was prepared from the plasmid generated for sequencing the transposon insertion site within DRO5F11. Approximately 1 µg plasmid DNA was digested with EcoRI-HF (New England Biolabs, Ipswich, MA, USA) in a 25-µl reaction at 37°C. After 1 h, the reaction was stopped by using a QIAquick PCR Purification Kit (Qiagen, Venlo, Netherlands) according to the manufacturer's instructions and eluted with 40 µl water.

A culture of ES114 harboring pLosTfoX was grown overnight at 28°C with aeration in DMM containing 9.9 mM glucosamine and 2.5 µg/ml chloramphenicol. The culture was diluted 1:4 into fresh media and grown at 28°C. After 1.5 h, 500 µl culture was combined with DNA template described above and incubated at room temperature. After 30 min, 1 ml LBS was added to the mixture and grown at 28°C overnight with aeration. The culture was concentrated by centrifugation and the resulting pellet was resuspended in 150 µl LBS, which was spread onto LBS agar containing 5.0 µg/ml erythromycin and incubated at 28°C. An erythromycin-resistant colony was selected as DRO222 and further characterized as described in the main text.

Allelic exchange vectors. To construct the plasmid pTM327, which contains the Δ*luxPQ* allele that removes the codons corresponding to amino acids 5-372 of LuxP and 1-734 of LuxQ, the regions surrounding *luxPQ* were amplified from ES114 genomic DNA by PCR using primers luxPQ-5-XhoI-U/luxPQ-5-BamHI-L and luxPQ-3-BamHI-U/luxPQ-3-XbaI-L, digested by XhoI/BamHI and BamHI/XbaI, and cloned into the vector fragment of pEVS79 that had been digested with XhoI/XbaI.

To construct pTM375, which contains the Δ*sypK* allele that removes the codons corresponding to amino acids 13-465 of SypK, the regions surrounding *sypK* were amplified from ES114 genomic DNA by PCR using primers VF_A1030-5-SalI-U/VF_A1030-5-EcoRI-L and

VF_A1030-3-EcoRI-U/VF_A1030-3-NheI-L, digested by SalI/EcoRI and EcoRI/NheI, and cloned into the vector fragment of pEVS79 that had been digested with SalI/XbaI.

Introduction of the Δ*luxPQ* and Δ*sypK* alleles into ES114 was performed as previously described for other allele exchange vectors (Miyashiro *et al.*, 2010).

Construction of KV4829, KV5972, and KV6010, which contain deletions of *luxU*, *luxQ*, and *luxP* were generated using pVAR18, pVAR29, and pVAR30, respectively, as previously described (Ray and Visick, 2012).

Chromosomal integration vectors. To construct pTM239, which contains *gfp* downstream of the *qrr1* promoter, the *qrr1* promoter region was amplified from ES114 genomic DNA by PCR using primers qrr1-prom-KpnI-U/ qrr1-prom-SpeI-L, and the product was digested with KpnI/SpeI. The *gfp* gene was isolated by digesting pTM146 with SpeI/EcoRV. The *qrr1* promoter and *gfp* fragments were ligated to the vector fragment of pEVS107 that had been digested with SpeI, made blunt with T4 DNA polymerase, and digested with XbaI. Integration of pEVS107-derived plasmids into the Tn*7* site was performed as previously described (McCann *et al.*, 2003).

IPTG-inducible plasmids. Plasmids pTM367 and pTM368 contain *sypK* (*VF_A1030*) and *sypL* (*VF_A1031*), respectively, downstream of the IPTG-inducible P_{trc} promoter. To construct pTM367, *sypK* was amplified from ES114 genomic DNA by PCR using primers sypK-KpnI-U/sypK-SalI-L, digested with KpnI/SalI, and cloned into the vector fragment of pTM214 that had been digested with KpnI/SalI. To construct pTM368, *sypL* was amplified from ES114 genomic DNA by PCR using primers sypL-KpnI-U/sypL-SalI-L, digested with KpnI/SalI, and cloned into the vector fragment of pTM214 that had been digested with KpnI/SalI.

LuxQ variant plasmids. Plasmid pVAR48 contains the wild-type *luxQ* allele tagged with the FLAG epitope. To construct pVAR48, *luxQ* was amplified from ES114 genomic DNA by PCR

using primers 1314 and 1437, digested by XhoI/KpnI, and cloned into the vector fragment of pVSV105 that had been digested with SalI/KpnI.

Plasmids pVAR50 and pVAR51 contain the A216P and H378A alleles of *luxQ-FLAG*, respectively. To construct pVAR50 and pVAR51, pVAR48 was mutagenized using the Change-IT Multiple Mutation Site Directed Mutagenesis Kit (Stratagene, La Jolla, CA, USA) with primer sets 849/1425 and 849/1426, respectively. Mutations were confirmed by sequencing.

sypK plasmid. Plasmid pVAR70 contains the wild-type *sypK* allele tagged with the FLAG epitope. To construct pVAR70, *sypK* was amplified from ES114 genomic DNA by PCR using primers 1167 and 1580, digested by XbaI/KpnI, and cloned into the vector fragment of pVSV105 that had been digested with XbaI/KpnI. The clone was confirmed by sequencing. The *sypK-FLAG* allele was subcloned from the resulting vector using SalI/BglI, and cloned into the vector fragment of pKV282 that had been cut with SalI/BamHI.

Table S1. Primers used in this study.

Primer name	Primer Sequence
luxPQ-5-XhoI-U	GGCTCGAGGTAGCGATAACTACAGATACTTTA
luxPQ-5-BamHI-L	GTGGATCCGTTGCGCATTACAACCAACCAGAA
luxPQ-3-BamHI-U	GAGGATCCGAGAACGGGTTGCCACCTTTTGAC
luxPQ-3-XbaI-L	GATCTAGAGATGTTGCGATTATGGATTTACCC
VF_A1030-5-SalI-U	AGGTCGACGCAAAAGCCATTATGGTGTTTATC
VF_A1030-5-EcoRI-L	GGGAATTCTTTAGCATGGGTGATAAAGGAAAT
VF_A1030-3-EcoRI-U	CGGAATTCTCTCATCACTTTTCCTAAAAAAGA
VF_A1030-3-NheI-L	GGGCTAGCGGATTTACTCTTTTAATTGGTTTA
qrr1-prom-KpnI-U	GAGGTACCAGCCAACACATCAAAACCTG
qrr1-prom-SpeI-L	AAACTAGTGGTCAATATACCTATTGCAG
849	CCTGTGTGAAATTGTTATCCG
1167	CATACTAAGTGCGGCCGCCTATCATTAGATGAGGTGAAACAATGAG
1314	CTGGTGTAGAACACTAGTAATG
1425	GATAATAAGATATTACCATCAAGTGTAAGTTCC
1426	TTAGCAAGAATGAGTGCTGAAATAAGAACGCCT
1437	AAAAAGGTACCTTATTTATCATCATCATCTTTATAATCCGAGTCAAAAGGTGGCAACCCG
1580	GGTACCTTATTTATCATCATCATCTTTATAATCTAGTGTTTTTCCTGTTTGTTCTTTTTTAGG
sypK-KpnI-U	CGGGTACCTTAGATGAGGTGAAACAATGAGTC
sypK-SalI-L	CGGTCGACTCATAGTGTTTTTCCTGTTTGTTC
sypL-KpnI-U	GGGGTACCGAACAAACAGGAAAAACACTATGA
sypL-SalI-L	GGGTCGACTTACTCTTTTAATTGGTTTAATGC
rpoD-qPCR-U	AGAAACCGCTCCTGATGCTGATGA
rpoD-qPCR-L	TACTGGGTCAGTTGTACGGCCAAT
litR-qPCR-U	AGGCCTAGAACAAGGCTATCTCCA
litR-qPCR-L	TAGCGACAGAGACCTGAGCGATTT
sypA-qPCR-U	AAGGTGATATGGACGCCATCGGTT
sypA-qPCR-L	AGCGCCAATACCTGATGAATCT
sypF-qPCR-U	AAGGCCCAGGAGCACAAGTTGATA
sypF-qPCR-L	GCAGCTTCTGCTTCATTTCTGGCT
sypJ-qPCR-U	CAGCTCAACTTGATCGTCTTCGCT
sypJ-qPCR-L	TCCCGCAATGAGAGCTTCAAGGAT
sypK-qPCR-U	TGGCTTTCGAAGGAATGATTGCCG
sypK-qPCR-L	ATGGCCTGAAGTAATGCTCGACCT
sypL-qPCR-U	CTTGGCATCAACGCCTCATTGGTT
sypL-qPCR-L	TCCGCCTCGACTTTGTGTTGCTAT
sypM-qPCR-U	TGCTTTACGGTGGCTTACCCTTCT
sypM-qPCR-L	ATTCGTTCGCCCACTAAAGGTCGT
sypP-qPCR-U	TTGTTGTTGGTAATGCTGGGCGAC
sypP-qPCR-L	AGCCAGAACCTGCAAGCAGTAAGT

References

McCann, J., Stabb, E. V., Millikan, D. S. and Ruby, E. G. (2003) Population dynamics of *Vibrio fischeri* during infection of *Euprymna scolopes*. *Appl Environ Microbiol* **69:** 5928-34.

Miyashiro, T., Wollenberg, M. S., Cao, X., Oehlert, D. and Ruby, E. G. (2010) A single *qrr* gene is necessary and sufficient for LuxO-mediated regulation in *Vibrio fischeri*. *Mol Microbiol* **77:** 1556-67.

Ray, V. A. and Visick, K. L. (2012) LuxU connects quorum sensing to biofilm formation in *Vibrio fischeri*. *Mol Microbiol* **86:** 954-70.