

# Characterization of mixed-species biofilm formed by *Vibrio parahaemolyticus* and *Listeria monocytogenes*

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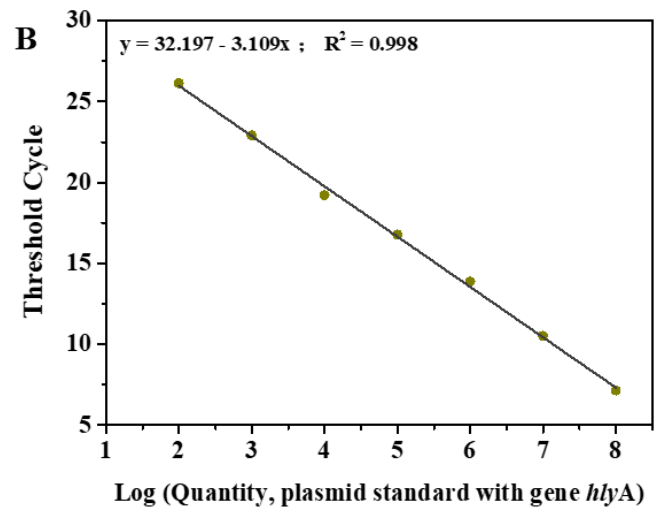
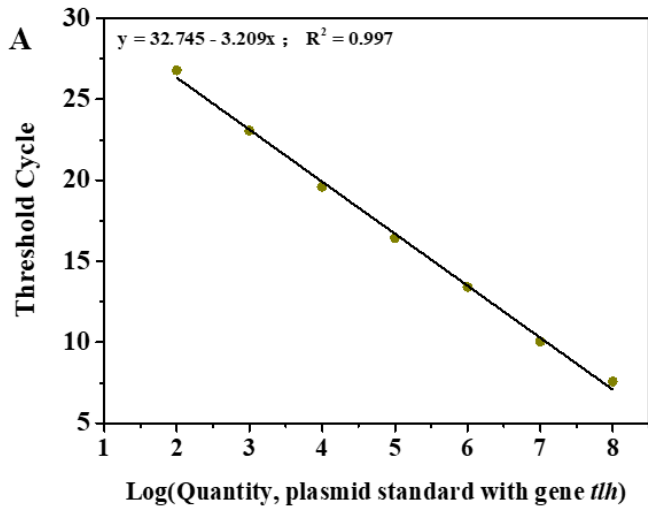
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**FIGURE S1: Standard curves for real-time qPCR in pure culture.** **A:** Standard curves for *V. parahaemolyticus* based on plasmid standard with *tlh*. **B:** Standard curves for *L. monocytogenes* based on plasmid standard with *hlyA*.

**Table S1 | Primer sequences for the RT-qPCR assay**

<b>Target gene</b>	<b>Nucleotide sequence (5'→3')</b>	<b>Reference</b>
<i>recA</i>	F - GCTAGTAGAAAAAGCGGGTG R - ATACGAGTGGTTGCTGTCATG	Ma et al., 2015
<i>mshA</i>	F - GGTTTCGTTTAGGTCACG R - CGTCGAAATGTCGGCGG	Shime-Hattori et al., 2006
<i>aphA</i>	F - ACACCCAACCGTTCGTGATG R - GTTGAAGGCGTTGCGTAGTAAG	Wang et al., 2013
<i>opaR</i>	F - TGTCTACCAACCGCACTAACC R - GCTCTTCAACTCGGCTTCAC	Zhang et al., 2016
<i>oxyR</i>	F - TCGTCAGCTAGAGGAAGG R - TGGTCGCGTAAGCAATGC	Chung et al., 2016
<i>Gap</i>	F-AAAGCTGGCGCTAAAAAAGTTG R-TTCATGGTTTACATTGTAAACGATTG	Mattila et al., 2011
<i>flaE</i>	F - CAGCAGGTTCCCCGACTTC R - CGGCCTTGTAGTGCTGCAT	This study
<i>motB</i>	F - TGCAAAAAAATTCGAACAAATGG R - CTGCCGCGCCTTCCT	This study
<i>degU</i>	F - GGAGGAGTAGTCATTATGGC R - ACTTCTGGTTGTTGGTAGCC	This study
<i>flaA</i>	F - GAAGGCATGACTCAAGCGCA R - GCAAGACCAGCAGCGTCATC	This study

## REFERENCES

- Chung, C.H., Fen, S.Y., Yu, S.C., and Wong, H.C. (2016). Influence of oxyR on Growth, Biofilm Formation, and Mobility of *Vibrio parahaemolyticus*. *Applied and Environmental Microbiology* 82(3), 788-796. doi: 10.1128/AEM.02818-15
- Ma, Y.J., Sun, X.H., Xu, X.Y., Zhao, Y., Pan, Y.J., Hwang, C.A., et al. (2015). Investigation of Reference Genes in *Vibrio parahaemolyticus* for Gene Expression Analysis Using Quantitative RT-PCR. *Plos One* 10(12), e0144362. doi: 10.1371/journal.pone.0144362
- Mattila, M., Lindström, M., Somervuo, P., Markkula, A., and Korkeala, H. (2011). Role of flhA and motA in growth of *Listeria monocytogenes* at low temperatures. *International Journal of Food Microbiology* 148(3), 177-183. doi: 10.1016/j.ijfoodmicro.2011.05.022
- Shime-Hattori, A., Iida, T., Arita, M., Park, K.S., Kodama, T., and Honda, T. (2006). Two type IV pili of *Vibrio parahaemolyticus* play different roles in biofilm formation. *Fems Microbiology Letters* 264(1), 89-97. doi: 10.1111/j.1574-6968.2006.00438.x
- Wang, L., Ling, Y., Jiang, H.W., Qiu, Y.F., Qiu, J.F., Chen, H.P., et al. (2013). AphA is required for biofilm formation, motility, and virulence in pandemic *Vibrio parahaemolyticus*. *International Journal of Food Microbiology* 160(3), 245-251. doi: 10.1016/j.ijfoodmicro.2012.11.004
- Zhang, Y.Q., Zhang, L.Y., Hou, S.N., Huang, X.X., Sun, F.J., and Gao, H. (2016). The Master Quorum-Sensing Regulator OpaR is Activated Indirectly by H-NS in *Vibrio parahaemolyticus*. *Current Microbiology* 73(1), 71-76. doi: 10.1007/s00284-016-1018-8