THE NATURAL FURANONE (*5Z*)-4-BROMO-5-(BROMOMETHYLENE)-3-BUTYL-2(*5H*)-FURANONE DISRUPTS QUORUM SENSING IN *VIBRIO HARVEYI* BY DECREASING THE DNA-BINDING ACTIVITY OF THE MASTER REGULATOR LUXR

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Our previous work showed that quorum sensing-disrupting furanones significantly increase the survival of brine shrimp infected with luminescent vibrios (Defoirdt *et al.*, 2006). This study aimed at getting a deeper insight in the molecular mechanism by which the natural furanone (*5Z*)-4-bromo-5-(bromomethylene)-3-butyl-2(*5H*)-furanone disrupts quorum sensing in *Vibrio harveyi*. Bioluminescence experiments with signal molecule receptor double mutants revealed that the furanone blocks all three channels of the *V. harveyi* quorum sensing system. In further experiments using mutants with mutations in the quorum sensing signal transduction pathway, the compound was found to block quorum sensing-regulated bioluminescence by interacting with a component located downstream of the Hfq protein.

Furthermore, reverse transcriptase realtime PCR with specific primers showed that there was no effect of the furanone on $luxR_{Vh}$ mRNA levels in wild type *V. harveyi* cells. In contrast, mobility shift assays showed that in the presence of the furanone, significantly lower levels of the $LuxR_{Vh}$ response regulator protein were able to bind to its target promoter sequences in wild type *V. harveyi*. Finally, tests with purified $LuxR_{Vh}$ protein also showed less shifts with furanone-treated $LuxR_{Vh}$, whereas the $LuxR_{Vh}$ concentration was found not to be altered by the furanone (as determined by SDS-PAGE). Therefore, our data indicate that the furanone blocks quorum sensing in *V. harveyi* by rendering the quorum sensing master regulator protein $LuxR_{Vh}$ unable to bind to the promoter sequences of quorum sensing-regulated genes.

The fact that the furanone affects the master regulator rather than selectively blocking one of the channels of the *V. harveyi* quorum sensing system is quite important with respect to possible practical applications since there seems to be a difference in the relative importance of the three channels for a successful infection of different hosts (Defoirdt *et al.*, 2005; Tinh *et al.*, 2007). Since the furanone blocks all three channels of the system at once by acting at the end of the quorum sensing signal transduction cascade, it will not be necessary to develop different furanone compounds to protect different hosts. In addition to this, human pathogens, including *Vibrio cholerae*, *Vibrio parahaemolyticus* and *Vibrio vulnificus*, have been shown before to contain $LuxR_{Vh}$ homologues (Milton, 2006).

References

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