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Quorum Sensing System and Virulence Regulation in *Vibrio vulnificus*

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Quorum sensing is a bacterial cell intercommunication system that controls multiple gene expressions in response to population density. It is now recognized as a widespread regulatory mechanism used by many Gram negative and Gram positive bacteria. Quorum sensing regulates specialized processes such as bioluminescence, cell division, competence, pathogenesis, conjugation, motility, biofilm formation, exopolysaccharide production, and antibiotic production when bacterial population reaches at a sufficient cell density. Quorum sensing bacteria synthesize, release, and detect to specific signaling molecules called autoinducers that accumulate in the environment as the bacteria grow. Autoinducers allow a bacterium to sense its own population density in a given environment and bacterial recognition of autoinducers can induce target gene expressions. The prototypic quorum sensing bacteria *Vibrio harveyi* has two independent density sensing system, signaling system 1 and 2, and each is composed of a sensor-autoinducer pair. The signaling system 1 is composed of the autoinducer 1 (AI-1) N-3-hydoroxybutanoyl-L-homoserine lactone and its relevant sensor 1 LuxN. The signaling system 2 is composed of the sensor 2 LuxPQ and the autoinducer 2 (AI-2) whose structure remains unknown. AI 1-type quorum sensing systems in various bacteria, such as *Vibrio fischeri*, *Pseudomonas aeruginosa*, and *Agrobacterium tumefaciens*, have been well characterized and correlated with various bacterial physiologies and virulence factors. The signaling system 1 is a highly specific system proposed to be used for intraspecies communication. In 1999, the AI-2 synthase gene (*luxS*) of *V. harveyi* was first identified and *luxS* homologues have been discovered in many bacterial species including *E. coli*, *Salmonella typhimurium*, *V. cholerae*, and *Helicobacter pyroli*. Unlike AHL quorum sensing that showes maximal activity during stationary phase, the LuxS quorum sensing showes maximal signaling activity in mid exponential phase and the signal disappearance in late stationary phase. The AI-2s produced by various Gram-negative bacteria cross-react over the genus/species barrier. Also the LuxS system is affected by multiple environmental factors such as carbon sources, pH, and/or osmolarity. The species non-specific LuxS quorum sensing system is suggested to participate in monitoring the environment for the presence of other species of bacteria and plays a certain function at low cell density. The LuxS quorum sensing system might play important roles in regulating virulence factors since *luxS* gene of *E. coli* was mutated in the nonvirulent laboratory DH5a strain. Furthermore a recent report presented that the *luxS* quorum sensing system controls expression of the type III secretion gene transcription and protein secretion in enterohemorrhagic and enteropathogenic *E. coli*. Some pathogenic bacterial species

seems to have only the LuxS quorum sensing system. *V. cholerae*, whose genome project was recently completed, does only have the LuxS system, not the HSL mediated signaling system 1.

We tested whether *V. vulnificus* produces signaling molecules (AI 1 and/or 2) that respond to *Vibrio harveyi* quorum sensing system 1 and/or 2. Although we could not prove the presence of signaling system 1 in *V. vulnificus*, we found that the bacterium has a signaling system that induces luminescence expression in *V. harveyi* through the signaling system 2. Maximal AI-2 activity was observed during mid exponential to early stationary phase and disappeared in late stationary phase when *V. vulnificus* was grown in 2.5%-NaCl heart infusion broth. *V. vulnificus* produced an increased signaling activity when it was cultured in the presence of glucose (0.5%) and low pH (pH 6.0). Using a cosmid library of *V. vulnificus* type strain ATCC 29307, we have cloned and sequenced the *luxS* gene of *V. vulnificus* (Vv-*luxS*), the autoinducer 2 synthase gene for signaling system 2, which is a homologue of the *luxS* gene of *V. harveyi* (Vh-*luxS*). To investigate the pathogenetic role of *luxS*, a *luxS* deletion mutant of MO6-24/O strain was constructed. The Vv-*luxS* mutant showed an increase in the hemolysin production and a delay in the protease production. Increased hemolysin activity and decreased protease activity were restored to isogenic wild type level by complementation with wild type Vv-*luxS* allele on a plasmid and wild type logarithmic phase spent media. Transcriptional activities of the exotoxin hemolysin (*vvhA*) and protease (*vvpE*) genes were also observed in the *luxS* mutant by using chromosomal *PvvhA*::*lacZ* and *PvvpE*::*lacZ* transcriptional reporter strains. In the reporter strains, transcriptions of *vvhA* and *vvpE* increased and decreased by the mutation, respectively. The mutation resulted in an attenuation of lethality to mice. LD50 increased by 10-fold and the time required for the death of infected mice was significantly delayed in the *luxS* mutant. These results suggest that harmonious expression of virulence factors in *V. vulnificus* is regulated by the LuxS quorum sensing system.