

Monitoring bacterial cell-to-cell communication “quorum sensing” using a BMG LABTECH microplate reader

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- Quorum sensing is a phenomenon allowing bacteria to adapt to environmental changes
- Effects on quorum sensing were investigated by measuring bioluminescence and bacterial growth
- The FLUOstar® OPTIMA was used to measure absorbance and luminescence in parallel

Introduction

Bacteria can monitor and respond to changes to environmental conditions via a cell density process known as quorum sensing (QS). Bacteria uses this process to monitor their community by producing, detecting and responding to low molecular mass signal molecules, called autoinducers (AI). When the cell density increases the concentration of these signaling molecules will also increase. Once the accumulation of these molecules reaches a threshold, bacteria are collectively able to regulate gene expression and therefore cooperatively regulate their metabolic behavior. Quorum sensing has been shown to regulate a variety of processes in bacteria. These include bioluminescence and symbiosis in *Vibrio fischeri*, expression of virulence genes in *Pseudomonas aeruginosa*, expression of virulence, surface proteins and biofilm formation in *Escherichia coli*, and biosynthesis of extracellular polymeric substances and pathogenicity in *Erwinia stewartii*. Two major types of quorum sensing molecules (QSMs) have been widely described in literature. The commonest QSM used by Gram-negative bacteria are known as N-acyl homoserine lactones (HSLs) whilst Gram-positive bacteria use amino acids and short peptides (oligopeptide) as their AIs.

Quorum sensing was first described in *V. fischeri*, a Gram-negative bacteria. At low cell density, *Vibrio fischeri* is non-bioluminescent, but when the concentration increases (high cell density), the organism is bioluminescent. The molecular basis for regulation of bioluminescence in *V. fischeri* via quorum sensing has been well studied. The gene cluster responsible for light production consists of eight genes (*luxA-E*, *luxG*, *luxI*, and *luxR*) (Figure 1).

The regulator proteins responsible for quorum sensing

in this organism are proteins encoded by *luxI* and *luxR*. *luxI* encodes the enzyme AHL synthase, which catalyses the reaction involved in the biosynthesis of HSLs known as N-3-oxo-hexanoyl-L-homoserine lactone [3-oxo-C6-HSL]. *luxR* encodes the protein which binds to the AI and also activates the *luxA-E* and *luxI* operons.

The aim of this study was to elucidate the effect of changes in environmental condition such as growth media as well as the addition of exogenous homoserine lactone on 3-oxo-C6-HSL on the growth and bioluminescence in *V. fischeri*. A BMG LABTECH microplate reader was used to measure absorbance and luminescence in script mode.

Materials & Methods

Bacteria strain and Media

V. fischeri ESR1 and its mutants were kindly supplied by Prof Edward G. Ruby. The ESR1 variants include a signal-negative mutant that does not synthesize the 3-oxo-C6-HSL signal but can still respond to exogenous 3-oxo-C6-HSL, [*V. fischeri* KV240] and a signal-blind strain that produces 3-oxo-C6-HSL but do not respond to 3-oxo-C6-HSL [*V. fischeri* KV267].

All *V. fischeri* strains were grown in Luria-Bertani salt media (LBS), which contains 1% (wt/vol) tryptone, 0.5% (wt/vol) yeast extract (wt/vol), 2% NaCl (wt/vol), and 0.3% (vol/vol) glycerol in 50 mM Tris-HCl (pH 7.5) or in seawater tryptone [SWT] which contains 0.5% (wt/vol) tryptone, 0.3% (wt/vol) yeast extract, and 0.3% glycerol (vol/vol) in 70% seawater.

Evaluation of *V. fischeri* growth and bioluminescence under different media and concentrations of homoserine lactone

The growth and bioluminescence of all *V. fischeri* strains were monitored using a multi-mode plate reader from BMG LABTECH. Briefly, overnight cultures of *V. fischeri* strains grown in the above media at 28°C were inoculated to fresh media (1:500 dilution) with or without the addition of exogenous 3-oxo-C6-HSL. 200 µL of diluted cells (quadruplicate) were then transferred into a white 96 well clear bottom microplate (Greiner Bio-One).

The instrument incubation temperature was set at 28°C with continuous shaking at 300 rpm. The OD₆₀₀ and luminescence were then measured every hour for at least 16 h. The *V. fischeri* bioluminescence reading was reported as relative light units (RLU) divided by OD₆₀₀.

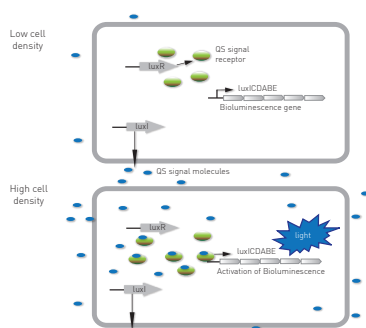


Fig. 1: The HSL-dependent LuxI/LuxR regulatory system of *V. fischeri*.



Results & Discussion

The increase in cell number over time for *V. fischeri* strains, grown in LBS and SWT can be seen in figure 2. The growth curves represent a typical growth curve under batch conditions with two clearly distinct phases i.e. exponential 2-8 h and onset of stationary phase after 8 h.

There was no significant difference between each strain, suggesting that quorum sensing in *V. fischeri* does not promote or inhibit growth.

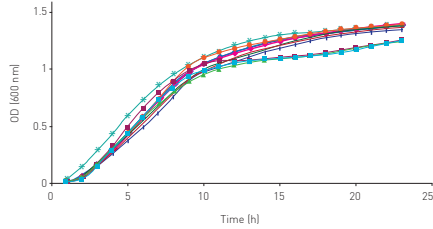


Fig. 2: Batch growth curve of *V. fischeri* strains grown in LBS and SWT with and without addition of 10 μ M 3-oxo-C6-HSL.

The expression of bioluminescence by *V. fischeri* ESR1 cultivated in LBS and SWT (with or without 3-oxo-C6-HSL) can be seen in figure 3 and 4 respectively. For *V. fischeri* ESR1, bioluminescence was higher in SWT than in LBS. Earlier activation of bioluminescence was observed when exogenous 3-oxo-C6-HSL was added to *V. fischeri* ESR1 at the onset of growth. For *V. fischeri* KV240, bioluminescence was only observed when exogenous 3-oxo-C6-HSL was added at the onset of growth. This confirms that the strain lacks the ability to produce endogenous 3-oxo-C6-HSL and can only express bioluminescence upon addition of exogenous 3-oxo-C6-HSL. As expected *V. fischeri* KV267, did express bioluminescence with or without the addition of exogenous 3-oxo-C6-HSL since it lacks the ability to respond to the signal molecule.

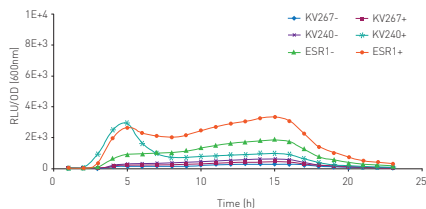


Fig. 3: Bioluminescence expression of *Vibrio fischeri* strains grown in LBS [+ means addition of 10 mM 3-oxo-C6-HSL; - means no 3-oxo-C6-HSL].*

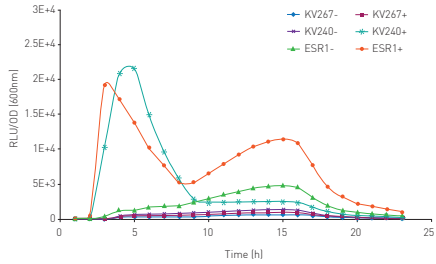


Fig. 4: Bioluminescence expression of *V. fischeri* strains grown in SWT [+ means addition of 10 μ M 3-oxo-C6-HSL; - means no 3-oxo-C6-HSL].

Conclusion

In this study, we show that BMG LABTECH microplate readers are a useful tool for understanding quorum sensing in bacteria. The instrument is able to monitor microbial growth and bioluminescence in parallel.

