Quorum sensing triggers stochastic expression of an asocial behavior in the early phase of biofilm development in *Pseudomonas putida*

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The Quorum-sensing Paradigm

Multiple Gene Expression

- Bioluminescence
- Virulence
- Secondary Metabolite Production
- Motility and Swarming
- Conjugation
- Biofilm Formation
- Growth Inhibition
- Pathogenicity

Amplification Loop

Lux box
Biofilms provide heterogeneous niches
QS is considered a social behavior

- QS often coordinates the release of beneficial public goods (hydrolytic enzymes, biosurfactants, toxins, biofilm matrix material) at the group level.

- Consequently, Many of the QS-controlled traits generate benefits to other cells in the community.

- QS-dependent regulation restricts the expression of costly cooperative behaviors to conditions, where they are most beneficial, which is the case at high cell density.

- The assumption that QS is a social behavior is supported by the demonstration of social cheating in test tubes and infections and is in agreement with the isolation of QS-negative strains in natural populations.
Sociomicrobiology: the connections between quorum sensing and biofilms

Matthew R. Parsek and E.P. Greenberg

.....we introduce the term sociomicrobiology, meaning ‘investigations of any group-behaviors of microbes’
Minor or no differences were observed in other studies:
Stoodley et al., 1999 Biofilms, 323-30
Heydorn et al., 2002; AEM 68, 2008-17
Purevdorj et al., 2002; AEM 68, 4457-64

BUT:
Quorum sensing in *Pseudomonas putida* IsoF
Identification of putative PpuR binding sites (*ppu* boxes)

Steidle et al., AEM 67:5761-70; 2001
**P. putida** IsoF produces mainly 3-oxo-C10-HSL

*E. coli* MT102(pSB403)  
*F*117(pKR-C12)
Expression of \textit{ppulI} is positively autoregulated

**Amount of AHLs in supernatant**

**ppul\_::\textit{luxAB} fusion**
The \textit{ppu} quorum-sensing locus of \textit{Pseudomonas putida IsoF}

$ppu$-box

\begin{itemize}
\item \textit{suhB}
\item \textit{ppuI}
\item \textit{rsal}
\item \textit{ppuR}
\item \textit{ppuA}
\end{itemize}

\hspace{1cm}

\begin{itemize}
\item \textit{secF}
\item \textit{secD}
\end{itemize}

\hspace{1cm}

$ppu$-box

\hspace{1cm}

\begin{itemize}
\item \textit{PA3819}
\item \textit{P. aeruginosa PAO1}
\item \textit{P. putida KT2440}
\item \textit{P. putida IsoF}
\item \textit{P. putida PCL1445}
\item \textit{P. putida WCS358}
\end{itemize}

$3$-oxo-$C10$-HSL

\hspace{1cm}

\begin{itemize}
\item \textit{PpuR}
\end{itemize}

\hspace{1cm}

\begin{itemize}
\item \textit{Target genes?}
\end{itemize}
The *ppu* locus of *P. putida* IsoF controls biofilm formation

Steidle et al., AEM 67:5761-70; 2001
The use of GFP allows detection of AHLs in real time and at the single cell level.
Detection of AHLs by the aid of Gfp-based sensors

<table>
<thead>
<tr>
<th>Strains to test</th>
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<td>Biosensor</td>
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0 µM OHHL

1 µM OHHL
Induction of QS in *P. putida* IsoF biofilms

Switching QS off

ON culture

OD < 0.3

Flow cell inoculum OD 0.01

Proportion of QS induced population
Quorate cells flee out of microcolonies
Quorate cells flee out of microcolonies
Older microcolonies collapse, the biofilm spreads out
What is the molecular basis of QS-triggered biofilm escape?

Lessons from *P. putida* PCL1445
P. putida PCL1445 produces a powerful biosurfactant

Kuiper et al., Mol. Microbiol. 51:97-113; 2004
*P. putida* PCL1445 produces the biosurfactants putisolvin A & B

Kuiper et al., Mol. Microbiol. 51:97-113; 2004
Pseudomonas putida strain PCL1445 putisolvin biosynthetic gene cluster

Dubern et al., Microbiology 154, 2070–2083; 2008

Condensation domain  Adenylation domain  Thiolation domain  Thioesterase domain
Influence of purified putisolvin I on biofilm formation of *P. putida* PCL1445 and its mutant PCL1436

Kuiper et al., Mol. Microbiol. 51:97-113; 2004
Construction of a conditional putisolvin producing strain in *P. putida IsoF*

Chromosome of *P. putida IsoF*

**pSC200-Gm::partial psoA**

- **DHFR**
- **RhaR**
- **RhaS**
- **Shine-Dalgarno**
- **Gm**
- **oriT**

**psoR**

**psoA**
Production of putisolvin is dependent of \( psoA \) and is regulated by the PpuI/PpuR system.

<table>
<thead>
<tr>
<th>Medium</th>
<th>IsoF (wt)</th>
<th>F117 (( ppul ))</th>
<th>PL11 (( psoA ))</th>
<th>PL2 (( cpsoA ))</th>
<th>PL2 (( cpsoA )) + rhamnose</th>
</tr>
</thead>
</table>
Production of putisolvin reduces surface tension

![Graph showing the relationship between surface tension and OD600nm for P. putida PL2 (cpsA)]
Swarming motility of *P. putida IsoF* is dependent on the production of putisolvin
Putisolvin production is regulated by the PpuI/PpuR system
Expression of \textit{psoA} is regulated by the PpuI/PpuR system

\textbf{Diagram:} 
- IsoF (\textit{wt})
- F117 (\textit{cepI})
- GC3 (\textit{cepR})

\textit{psoA} transcription (RFUs/OD\textsubscript{600})

\begin{itemize}
  \item IsoF (\textit{wt})
  \item F117 (\textit{cepI})
  \item GC3 (\textit{cepR})
\end{itemize}

\textbf{Legend:} + AHL
Two PpuR-regulated gene loci are required for putisolvin biosynthesis
Putisolvin production affects biofilm structural development
Expression of \textit{psoA} within a \textit{P. putida} IsoF biofilm

\begin{figure}
\centering
\includegraphics[width=\textwidth]{images}
\end{figure}
BUT

QS-triggered biofilm escape can only work if putisolvinins are not a public good!
Putisolvin is a private good
Putisolvin is a private good
Cells attach and form a microcolony

Stochastic induction of QS in few cells that begin to produce putisolvin

Putisolvin-producing cells migrate out of the microcolonies

Most cells in the microcolony are QS-positive
Because of massive putisolvin production microcolonies collapse

In the very early stage QS appears to trigger biofilm escape
In later stage of biofilm development it follows the textbook concept

QS uninduced, Pso negative
QS induced, Pso positive
**QS in *P. putida* IsoF: Heretic conclusions**

- AHL signal production is stochastically expressed in only a fraction of the cells in young biofilms.

- AHL production in one cell did not induce AHL production in its neighboring cells and thus is primarily used for self- and not for cross-induction.

- Putisolvins biosurfactants remained associated with the envelope of the producing cell, and therefore do not represent public goods.
Expression of \textit{psoA} within a \textit{P. putida} IsoF biofilm
Putisolvin production is regulated by complex regulatory circuitry

Dubern et al., JB 187:5967-76; 2005
Dubern et al., JB 188:2898-06; 2006
Dubern et al., FEMS Microbiol lett. 263:169-75; 2006
Bertani et al., BMC Microbiology 7:71; 2007
Fig S1
Fig S2
Flow chamber setup

Medium

Pump

Bubble traps

Flow-cell

Effluent

Cover slip

Flow
Induction of QS in *P. putida* IsoF biofilms
Expression of *ppuA* is quorum sensing-regulated

**Induction by AHLs**

**Inhibition by furanones**

Steidle et al., AEM 67:5761-70; 2001
Influence of putisolvins on biofilm formation and formed biofilms of *Pseudomonas fluorescens* WCS365 and *P. aeruginosa* PA14

Cells of *P. fluorescens* WCS365 and *P. aeruginosa* PA14 were incubated in microtitre plates in M63 medium with the following additions (0.5 µl): water (black bars), water and methanol/acetonitrile, 1:1, v/v (grey bars), with PCL1445 culture supernatant extracts (blocked bars), with putisolvin I (arced bars) or putisolvin II (white bars) dissolved in methanol/acetonitrile, 1:1.

A. Biofilms were analysed after 10 h of incubation with the additions (18 µM putisolvin) applied at time 0.

B. Biofilms were allowed to form for 7 h without additions after which the medium was replaced by fresh M63 medium with additions (28 µM putisolvin). Biofilms were analysed 1.5 h after the application. The experiment was performed in triplicate.

Kuiper et al., Mol. Microbiol. 51:97-113; 2004