Regulation of biofilm formation in *Pseudomonas* and *Burkholderia* species

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Localized biofilm dispersal in developing *Pseudomonas putida* biofilm.

Day 2                                     Day 3                                          Day 5

CLSM shadow projection                              CLSM optical section                                    CLSM shadow projection


Global dispersal of *P. putida* biofilm in response to carbon starvation.

0 min                                        5 min                                       15 min


Global dispersal of *P. putida* biofilm in response to carbon starvation.

*P. putida* lapG mutant biofilm does not disperse in response to carbon starvation

0 min                                        5 min                                       15 min

Biofilm formation of the *P. putida* wild type and lapG mutant in microtiter trays.


**Organization of the lap cluster in *P. putida***:

Comparative sequence analysis suggested that:

- LapA is a large adhesive surface associated protein
- LapD is a membrane spanning signaling protein with a degenerate EAL domain
- LapG is a periplasmic proteinase


c-di-GMP signaling regulates biofilm formation in various bacteria

2 GTP

GGDEF

EAL

c-di-GMP

Biofilm formation

Hypothesis

High c-di-GMP level

Low c-di-GMP level


Biofilm phenotypes of *P. putida* WT, lapG, lapA, and lapAG.

WT

lapG

lapA

lapAG


Biofilm phenotypes of *P. putida* WT, lapG, lapD, and lapDG.

WT

lapG

lapD

lapDG

Induction of an EAL domain protein in \textit{P. putida} leads to biofilm dispersal.

\[ pYhjH \]

\[ pYhjH\text{mut} \]


Induction of an EAL domain protein in \textit{P. putida} \textit{lapG} does not induce biofilm dispersal.

\[ WT \]

\[ \textit{lapG} \]


Navarro et al 2011, \textit{PLoS Biology} 2 | e1000588

\[ \textit{P. aeruginosa}\textit{'s PA1333 and PA1334 are homologous to } \textit{P. putida}\textit{'s lapD and lapG but there is no apparent target for PA1334.} \]

Partial alignments of the amino acid sequences of the \textit{LapD} (A) and \textit{LapG} (B) homologs from \textit{P. putida KT2440}, \textit{P. fluorescens PF0-1} and \textit{P. aeruginosa PAO1} (PA1433 and PA1434).

\[ \textit{A:} ^* \text{Degenerate GGDEF and EAL motifs.} \# \text{Functionally important residues as described by Newell et al. (2009), and Navarro et al. (2011). Black and grey shading denotes identical and similar residues, respectively, across all three sequences.} \]

\[ \textit{B:} ^* \text{The catalytic triad as predicted by Ginalski et al. (2004).} \# \text{Functionally important calcium binding residues as described by Boyd et al. (2014). Black and grey shading denotes identical and similar residues, respectively, across all three sequences.} \]

Biofilm formation by \textit{P. putida} \textit{lapD} and \textit{lapG} mutants complemented with homologous genes from \textit{P. aeruginosa PAO1}.

The bars indicate amount of biofilm after 6 hours (white bars) and 20 hours (grey bars).

Biofilm formation by \textit{P. aeruginosa PAO1} \textit{WT} and \textit{lapG} mutant.

The bars indicate amount of biofilm after 6 hours (white bars) and 20 hours (grey bars).
Deletion of lapG in a *P. aeruginosa* strain with increased levels of c-di-GMP results in a hyper-clumping phenotype.

### List of transposon mutants in the wspF lapG double deletion background identified as deficient in the hyper-aggregating phenotype

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-7, 2-59</td>
<td>cdrA</td>
</tr>
<tr>
<td>2-42</td>
<td>cdrB</td>
</tr>
<tr>
<td>1-6</td>
<td>pslA</td>
</tr>
<tr>
<td>2-54</td>
<td>pslB</td>
</tr>
<tr>
<td>1-11</td>
<td>lapG</td>
</tr>
<tr>
<td>2-6</td>
<td>fenv</td>
</tr>
<tr>
<td>1-3, 1-5</td>
<td>wspA</td>
</tr>
<tr>
<td>2-4</td>
<td>wspD</td>
</tr>
<tr>
<td>2-13</td>
<td>wspF</td>
</tr>
<tr>
<td>2-3</td>
<td>PA243940 intergenic region</td>
</tr>
<tr>
<td>1-1</td>
<td>capA3</td>
</tr>
<tr>
<td>2-2, 2-40</td>
<td>pslF</td>
</tr>
</tbody>
</table>

The hyper-clumping phenotype of the wspF lapG strain is dependent on CdrA.

Overexpression of cdrAB leads to hyper-clumping, and this can be resolved by simultaneous overexpression of lapG.

Western blot detection of CdrA in whole-cells and supernatant.
The LapG protein plays a role in P. aeruginosa biofilm formation by controlling the presence of the CdrA adhesin on the cell surface.

Regulation of biofilm formation in B. cenocepacia

B. cenocepacia forms wrinkly colonies and thick pellicles in response to increased c-di-GMP levels.

Transposon mutagenesis in B. cenocepacia/pYedQ, and screening for smooth colony formers identified the bcam1349 mutant.

The bcam1349/pYedQ strain is deficient in biofilm formation in flow-chambers.

The bcam1349 mutant is deficient in biofilm formation in flow-chambers.
Comparative sequence analysis suggested that Bcam1349 is a transcriptional regulator of the CRP/FNR family, of which a well-known example is the *E. coli* protein Crp.


Transposon mutagenesis identified *B. cenocepacia* mutants that do not form wrinkly colonies and thick pellicles in response to over-expression of Bcam1349


*COMSTAT* biomass quantification of 3-day-old biofilms before and after SDS treatment. Each average and standard deviation value originates from analysis of 12 CLSM image stacks.

**B. cenocepacia Bcam1330-1341 cluster mutants do not form wrinkly colonies in response to over-production of c-di-GMP.**

qRT-PCR demonstrates that expression of the Bcam1330 and Bcam1331 genes is increased in response to over-production of Bcam1349 ( ), or c-di-GMP ( )

EMSA analysis shows that Bcam1349 binds to the Bcam1330 and Bcam1331 promoter regions in a manner that is stimulated by c-di-GMP

Bcam1330 and Bcam1331 promoter probe DNA was incubated with purified Bcam1349 protein in the presence of c-di-GMP as indicated.

**c-di-GMP-mediated regulation of B. cenocepacia H111 biofilm formation**

High c-di-GMP level

Bcam1330

Bcam1331

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