Small molecule mediated autoinduction of antibiotic biosynthesis in
the plant pathogen Erwinia carotovora.

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Bacteria have evolved sophisticated sensing mechanisms which
facilitate adaptation to fluctuating environmental conditions.
Commonly these sensory systems consist of a histidine protein kinase
which senses changes in a specific environmental parameter and
transmits the information to the regulator protein by phosphorylating
it. However not all types of sensor-regulator circuits relay
information via phosphoryl transfer. The lux operon which consists of a
bioluminescent phenotype on Vibrio fischeri includes luxR, the
product of which has previously been characterized as a member of the
response regulator superfamily [1]. luxR responds to a small
diffusible signal molecule, N-(3-oxohexanoyl)-L-homoserine lactone
(OHHL) the biosynthesis of which is directed by the luxI gene
product (Fig. 1(i)). Characteristically, the OHHL-luxR mediated
induction of bioluminescence is cell density dependent and provides a
mechanism by which a strong co-ordinated response can be
achieved by a population of bacterial cells i.e. it represents an
intercellular communication device [1,2].

We have recently demonstrated that the plant pathogen Erwinia
carotovora also employs OHHL in the regulation of both the
synthesis of the β-lactam antibiotic 1-carbapen-2-em-3-carboxylic
acid and exoenzyme virulence determinants [3-5] (Fig. 1(ii)). In this
paper we: (i) demonstrate the temporal relationship between OHHL
production, carbapenem biosynthesis and cell population density, (ii)
characterise the Erwinia lux homologues, carl and (iii) provide
evidence to suggest that the expression of carl and hence carbapenem is autoinducible.

In V.fischeri, bioluminescence is regulated in a cell density
dependent manner mediated through the accumulation, to a critical
threshold concentration, of the inducer OHHL [2]. Since Erwinia
employs the same small molecule to regulate carbapenem antibiotic
biosynthesis, we wish to determine whether OHHL production is
constitutive throughout growth or exhibited the same cell density
dependency seen in V.fischeri. By transforming the carbapenem
producer strain E. carotovora G8101 with the recombinant lux sensor
pGEl3, we followed the in vivo production of OHHL by monitoring
the induction of bioluminescence. Results clearly demonstrate that
OHHL synthesis in Erwinia is cell density dependent [6].

Fig 1. Structures of (i) N-(3-oxohexanoyl)-L-homoserine lactone
(OHHL) and (ii) 1-carbapen-2-em-3-carboxylic acid.

To examine further the temporal relationship between cell density,
OHHL production and carbapenem biosynthesis, we devised HPLC-
based assays which permitted the direct, concurrent analysis of OHHL
and carbapenem in culture supernatants. When assayed in this
manner, OHHL is detected prior to the induction of carbapenem with
antibiotic synthesis only commencing once OHHL has reached a
critical level (around 0.5 μg/ml). This threshold concentration is in
close agreement with the concentration of exogenous OHHL required
to induce carbapenem in OHHL-negative Erwinia mutants [4]. Fig. 2
shows that addition of synthetic OHHL to G8101 at the time of
induction abolishes cell density dependency in that carbapenem
biosynthesis is induced almost immediately and antibiotic production
thereafter mirrors growth.

When a 2.5 kb Pstl fragment isolated from E.carotovora G8101
was introduced in trans into E.coli [pBS315] it was found to
complement the luxI deletion and restored the bioluminescent
phenotype. DNA sequence analysis of the Pstl fragment revealed the
presence of a single ORF of 648 bp, designated carl, which shared
significant homology at both the DNA [35%] and translated amino-
acid level (25%) with the V.fischeri luxI gene and the recently
described luxI homologues from Enterobacteria, Pseudomonas,
Agrobacterium and Yersinia [6-8].

Table 1. Influence of OHHL on the activation of a carl-lacZ fusion
in an OHHL-negative Erwinia carotovora mutant MB2

<table>
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<tr>
<th>Strain</th>
<th>β-galactosidase activity (Miller units)</th>
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<tbody>
<tr>
<td>MB2[pJ9]</td>
<td>9</td>
</tr>
<tr>
<td>MB2[pCZI]</td>
<td>646</td>
</tr>
<tr>
<td>MB2[pCZ2]</td>
<td>9</td>
</tr>
</tbody>
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A 1.4kb EcoRI fragment containing the carI promoter region
was cloned in-frame into the promoterless lacZ fusion vector pJ9 in
both forwards (pCZ1) and reverse (pCZ2) orientations. The lacZ
fusions were transformed into the OHHL-negative Erwinia mutant
MB2. Strains were grown overnight at 30ºC in LB media and assayed
to determine β-galactosidase activity using ONPG as the substrate [9].

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