Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications.

Supplementary information

Attenuation of quorum sensing-mediated virulence in Gram-negative pathogenic bacteria: implications for the post-antibiotic era

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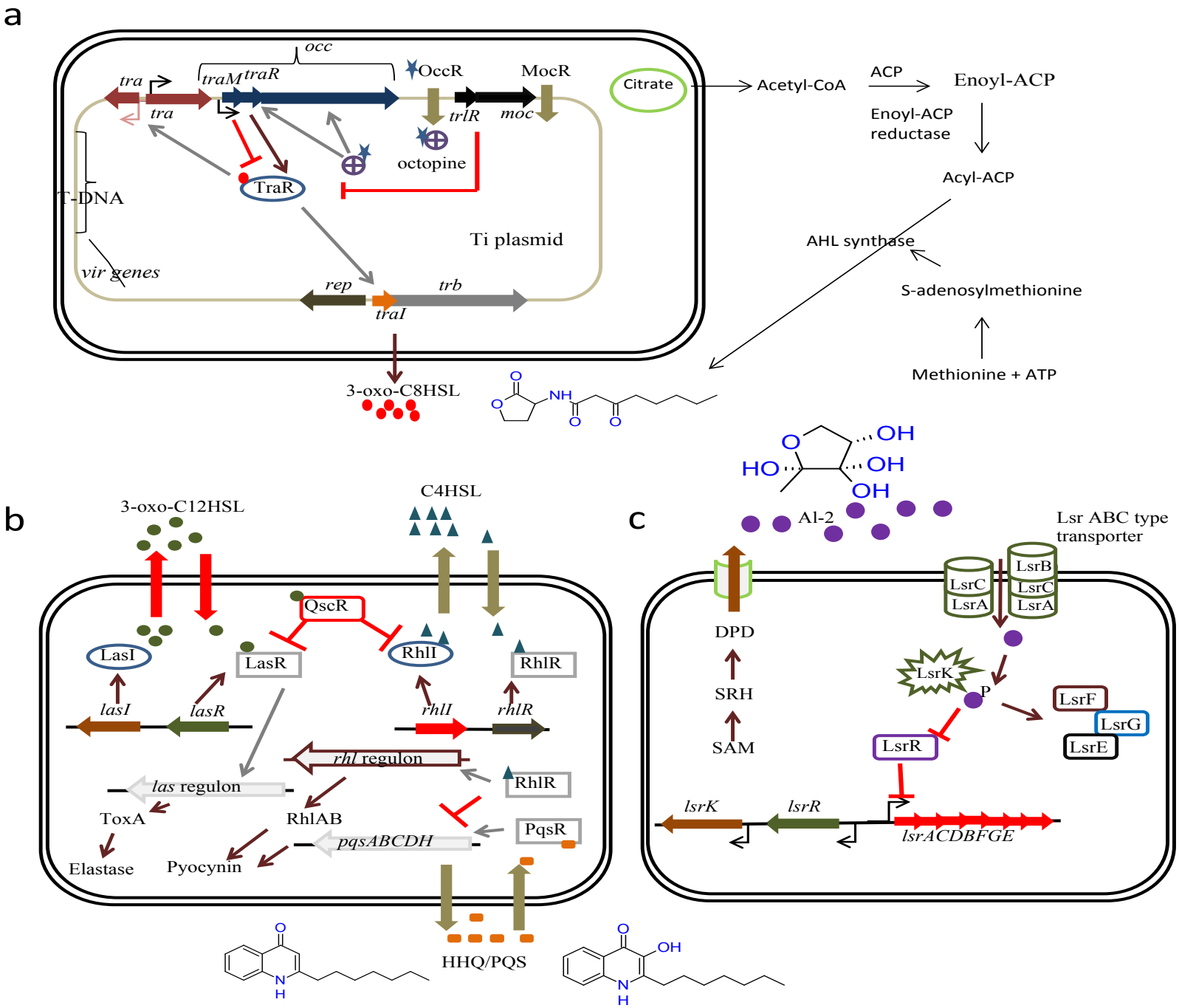
a
occ
tra
traM traR
OccR
MocR
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trlR
moc
octopine
TraR
T-DNA
Ti plasmid
vir genes
rep
trb
traI
3-oxo-C8HSL
Citrate
Acetyl-CoA
ACP
Enoyl-ACP
Enoyl-ACP reductase
Acyl-ACP
AHL synthase
S-adenosylmethionine
Methionine + ATP
b
3-oxo-C12HSL
C4HSL
QscR
LasI
LasR
RhlI
RhlR
lasI
lasR
rhlI
rhlR
rhl regulon
las regulon
RhlR
ToxA
RhlAB
PqsR
pqsABCDH
Elastase
Pyocynin
HHQ/PQS
c
AI-2
Lsr ABC type transporter
LsrC
LsrA
LsrB
LsrC
LsrA
DPD
SRH
SAM
LsrK
P
LsrF
LsrG
LsrE
LsrR
lsrK
lsrR
lsrACDBFGE

Supplementary Fig. 1. Gram-negative bacteria use acyl homoserine lactones (AHLs), autoinducers 2 and 3 as quorum-sensing (QS) signal molecules. At the threshold concentration, it expresses different types of products, those are responsible for acclimatising in different environmental conditions. (a) The TraI/TraR two-component QS system is present in *Agrobacterium tumefaciens*. TraI is synthesized 3-oxo-C_8HSL and the TraR is response regulator.[1,2] The OccR intracellular receptor induces octopine import. The *traR* is present at the distal end of the *occ* operon and activates in response to cognate opine. The TraR binds to 3-oxo-C_8-HSL and activates *tra* to further import 3-oxo-C_8-HSL, which induces the expression of *traI*. Tra is involved in the transfer of T-DNA.[3] The QS system in *A*. *tumefaciens* is also negatively controlled by TrlR and TraM proteins.[4,5] The figure also shows the general mechanism of AHL production. Fatty acid synthesis and *S*-adenosylmethionine intermediates are the substrates for the LuxI-type autoinducer synthases (1). Acyl-carrier-protein (ACP).

(b) QS pathways in *Pseudomonas aeruginosa.* There are two Lux-I-R homologue systems, Las-I-R and Rhl-I-R, that produce and detect the AHL signalling molecules 3-oxo-C_{12}.HSL and C_4-HSL, respectively.[6-8] The Las regulon controls *toxA, lasA, lasl, lasB* and *aprA* and the Rhl regulon controls *lasB*, *aprA*, *rpoS* and *rhlAB* expression.[6-8] The third QS system is the *Pseudomonas* quinolone signal (PQS). PQS systems consist of *pqsABCDH* structure genes along with a transcriptional regulator (*pqsR*) and the response effector (*pqsE*).[9] HHQ/PQS is involved in the condensation of anthranilate and a β-keto-fatty acid. LasR positively and RhlR negatively regulate the expression of the genes involved in the PQS system. The LurR-type protein, QscR, binds to 3-oxo-C_{12}HSL and subsequently inhibits both the *las* and *rhl* QS systems.[9-11]

(c) QS pathways in *Escherichia coli* and *Salmonella* Typhimurium. Autoinducer-2 is synthesised by LuxS and secreted out.[12-14] AI-2 is imported by the Lsr ABC-type transporter and imported AI-2 is phosphorylated by LsrK kinase.[15-16] Phosphorylated-AI-2 activates the QS circuit by binding to the repressor (LsrR), causing its release.[15] In the absence of phospho-AI-2, the repressor is bound to the *lsr* promoter region (*lsrACDBFG*) and prevents operon expression. LsrG is involved in the degradation of phospho-AI-2 and LsrE is a

putative sugar epimerase that is only present in *S.* Typhimurium.[15,17] S-adenosylmethionine (SAM), 4, 5-dihydroxy-2,3-pentanedione (DPD) and *S* -ribosylhomocysteine (SRH).

References

(1) L. Zhang, P. J. Murphy, A. Kerr and M. E. Tate, *Nature* 1993, **362**, 446–448.

(2) I. Hwang, L. Pei-Li, L. Zhang, K. R. Piper, D. M., Cook, M. E., Tate and S. K. Farrand, *Proc. Natl. Acad. Sci. USA.* 1994**, 91**, 4639–4643.

(3) K. R. Piper, S. Beck von Bodman and S. K. Farrand, *Nature* 1993, **362**, 448–450.

(4) C. Fuqua and S. C. Winans, *Mol. Microbiol.* 1996, **20**, 1199–1210.

(5) C. E. White and S. C. Winans, *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 2007**, 362**, 1135-48.

(6) M. J. Gambello and B. H. Iglewski, *J. Bacteriol.* 1991, **173**, 3000–3009.

(7) L. Passador, J. M. Cook, M. J. Gambello, L. Rust and B. H. Iglewski, *Science* 1993**, 260**, 1127–1130.

(8) J. P Pearson, K. M. Gray, L. Passador, K. D. Tucker, A. Eberhard, B. H. Iglewski and E. P. Greenberg, *Proc. Natl. Acad. Sci. USA* 1994, **91**, 197–201.

(9) L. A. Gallagher, S. L. McKnight, M. S. Kuznetsova, E. C. Pesci and C. Manoil, *J. Bacteriol.* 2002, **184,** 6472–6480.

(10) E. C Pesci, J. B Milbank, J. P. Pearson, S. McKnight, A. S. Kende, E. P. Greenberg and B. H. Iglewski, *Proc. Natl. Acad. Sci. USA* 1999, **96**, 11229–11234.

(11) J. F. Dubern and S. P. Diggle, *Mol. Biosyst.* 2008, **4**, 882– 888.

(12) S. Schauder, K. Shokat, M. G. Surette B. L. Bassler, *Mol. Microbiol.* 2001, **41**, 463–476.

(13) V. Sperandio, A. G. Torres, B. Jarvis, J. P. Nataro and J. B. Kaper, *Proc. Natl. Acad. Sci. USA* 2003, **100,** 8951–8956.

(14) K. Winzer, K. R. Hardie, N. Burgess, N. Doherty, D. Kirke, M. T. Holden, R. Linforth, K. A. Cornell, A. J. Taylor, P. J. Hill and P. Williams, Microbiology 2002, **148**, 909–922.

(15) M. E. Taga, S. T. Miller and B. L. Bassler, *Mol. Microbiol.* **50**, 1411–1427.

(16) B. M. Ahmer, *Mol. Microbiol.* 2004**, 52**, 933–45.

(17) K. B. Xavier and B. L. Bassler, 2005 *J. Bacteriol.* **187**, 238–48.