

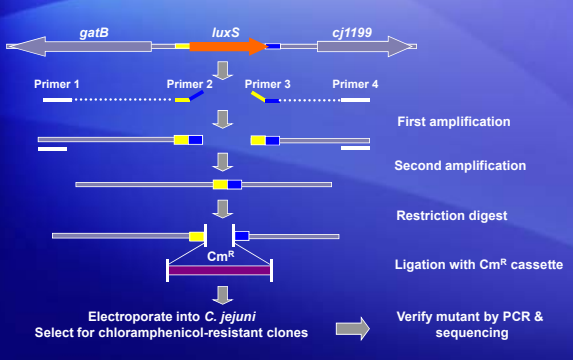


### Amino acid sequence alignment of LuxS

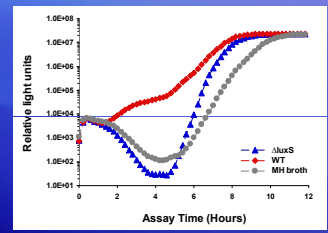
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E.coli      MPELLDSPTVDHTMEAPAVVAITNTFHGDALTVDLFCVFN EVMPE GIHTLEHLF 59
S.typhimurium MPELLDSFVVDHTMQAPAVVAITNTFHGDALTVDLFCVFN EVMPE GIHTLEHLF 59
V.harveyi    MPELLDSPTVDHTMQAPAVVAITNTFHGDALTVDLFCVFN DILGE GIHTLEHLF 59
H.influenzae MPELLDSPTVDHTMQAPAVVAITNTFHGDALTVDLFCVFN DIMSE GIHTLEHLF 60
C.jejuni     MPELLDSPTVDHTMQAPAVVAITNTFHGDALTVDLFCVFN DIMSE GIHTLEHLF 60
*****
E.coli      AGQENHINLNGNGVLIIDISPMGC TGFYNSLIGT FDEQAVADAM AAMADVAVQDQNI 119
S.typhimurium AGQENHINLNGNGVLIIDISPMGC TGFYNSLIGT FDEQAVADAM AAMADVAVQDQNI 119
V.harveyi    AGQENHINLNGNGVLIIDISPMGC TGFYNSLIGT FDEQAVADAM AAMADVAVQDQNI 119
H.influenzae AGQENHINLNGNGVLIIDISPMGC TGFYNSLIGT FDEQAVADAM AAMADVAVQDQNI 119
C.jejuni     AGQENHINLNGNGVLIIDISPMGC TGFYNSLIGT FDEQAVADAM AAMADVAVQDQNI 120
*****
E.coli      F.LIYDGGTYGNSAQ AQLARDAIRFRANSNL IAAIKKIQVHI - 170
S.typhimurium F.LIYDGGTYGNSAQ AQLARDAIRFRANSNL IAAIKKIQVHI - 170
V.harveyi    F.LIYDGGTYGNSAQ AQLARDAIRFRANSNL IAAIKKIQVHI - 171
H.influenzae F.LIYDGGTYGNSAQ AQLARDAIRFRANSNL IAAIKKIQVHI - 166
C.jejuni     F.LIYDGGTYGNSAQ AQLARDAIRFRANSNL IAAIKKIQVHI - 164
*****
    
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### Construction of $\Delta luxS$ mutant in *C. jejuni*

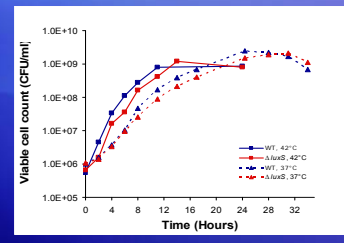


### LuxS-dependent AI-2 production



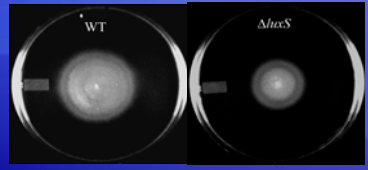
- Wild-type *C. jejuni* produces functional AI-2
- There is no AI-2 production in the *luxS* mutant
- LuxS is a key enzyme in the AI-2 biosynthesis pathway

### Growth curves of the wt and $\Delta luxS$ mutant of *C. jejuni*



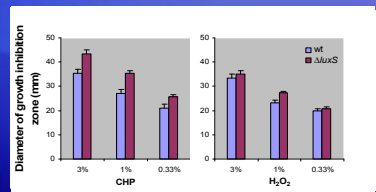
	37 °C	42 °C	37 °C	42 °C
WT	77 min	53 min	89 min	67 min
$\Delta luxS$ mutant	77 min	53 min	89 min	67 min

### Reduced motility in the $\Delta luxS$ mutant

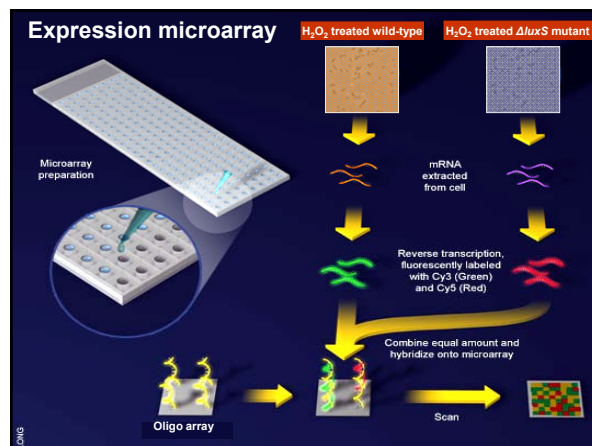
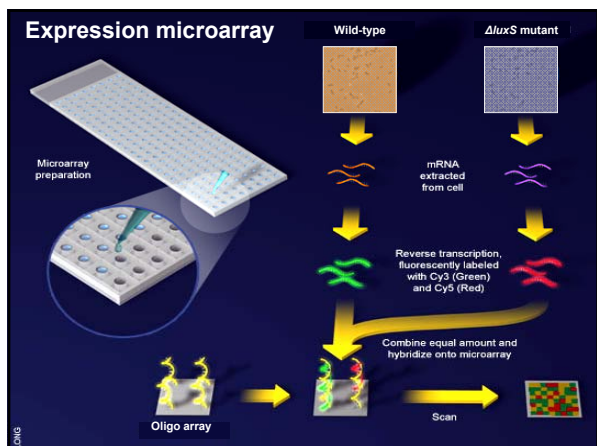


Halo diameter    35±1.22 mm                    28±1.58 mm

### Resistance of the wt and $\Delta luxS$ mutant to CHP and H<sub>2</sub>O<sub>2</sub>



CHP: cumene hydroperoxide  
H<sub>2</sub>O<sub>2</sub>: hydrogen peroxide



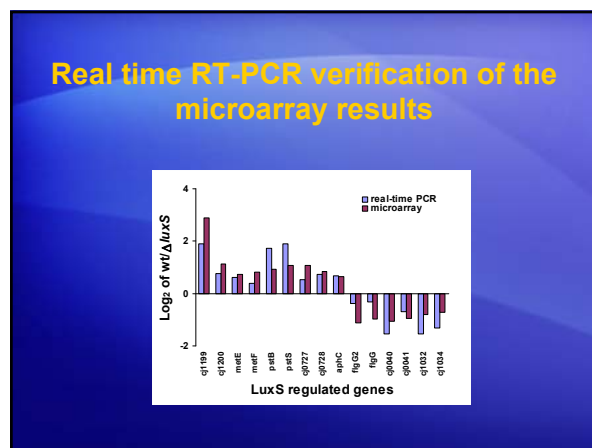
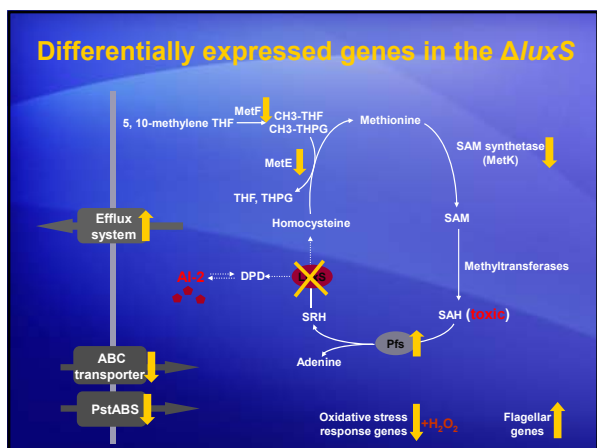
### Scanned microarray image

In both  $H_2O_2$  treated & untreated conditions:

- Triplicates of cell samples and chips were used.
- In each chip, there are triplicate sets of spotted oligos.
- Each set of oligos represents >1600 ORFs of *C. jejuni*.
- 9 replicate data was normalized and analyzed.
- The up/down-regulated genes were selected by 1.5 fold cut-off combining with P value < 0.05.

### Selected genes regulated by *luxS* with/without $H_2O_2$ treatment

Locus/Genes	Gene product	Fold change (wt/ <i>ΔluxS</i> )	
		+ $H_2O_2$	- $H_2O_2$
Cj1198 ( <i>luxS</i> )	S-ribosylhomocysteinase	331.6	195.5
Cj1199	putative iron/ascorbate-dependent oxidoreductase	7.4	11.1
Cj1200	putative periplasmic protein	2.2	3.2
Cj1201 ( <i>metE</i> )	homocysteine methyltransferase	1.66	0.95
Cj1202 ( <i>metF</i> )	5,10-methylenetetrahydrofolate reductase	1.8	1.36
Cj1096 ( <i>metK</i> )	S-adenosylmethionine synthetase	1.5	0.98
Cj0613 ( <i>pstS</i> )	possible periplasmic phosphate binding protein	2.1	2.9
Cj0615 ( <i>pstA</i> )	putative phosphate transport system permease protein	1.26	1.6
Cj0616 ( <i>pstB</i> )	phosphate ABC transporter, ATP-binding protein	1.9	2.1
Cj0727	ABC transporter, periplasmic substrate-binding protein	2.1	1.57
Cj0728	putative periplasmic protein	1.8	1.48
Cj0301 ( <i>modB</i> )	molybdenum ABC transporter	2.0	1.12
Cj1220 ( <i>groES</i> )	co-chaperonin	1.69	1.27
Cj0334 ( <i>ahpC</i> )	alkyl hydroperoxide reductase	1.56	1.03
Cj0779 ( <i>tpx</i> )	probable thiol peroxidase	1.61	1.08
Cj0117 ( <i>pfS</i> )	S-adenosylhomocysteine nucleosidase	0.66	0.68
Cj0697 ( <i>fgG2</i> )	putative flagellar basal-body rod protein	0.46	0.84
Cj0698 ( <i>fgG</i> )	flagellar basal-body rod protein	0.51	0.70
Cj0040	flagellar assembly protein	0.48	0.81
Cj0041	hypothetical protein	0.52	0.70
Cj0042 ( <i>fgD</i> )	putative flagellar hook assembly protein	0.49	0.63
Cj0043 ( <i>fgE</i> )	flagellar hook protein	0.58	0.64
Cj1031	putative outer membrane component of efflux system	0.76	0.74
Cj1032	putative membrane fusion component of efflux system	0.57	0.87
Cj1033	putative integral membrane component of efflux system	0.64	0.99
Cj1034c	possible dnaJ-like protein	0.61	0.71



## Summary

- In *C. jejuni*, AI-2 production is dependent on LuxS.
- The  $\Delta luxS$  mutant exhibits slightly slower growth, lower motility, and higher sensitivity to oxidative stress than those of the wt strain.
- Microarray analysis revealed that AI-2/LuxS regulates the expression of genes involved in AI-2 biosynthesis and methyl cycle, ABC transporter, membrane efflux system, stress responses, and cell motility.
- Expression of the oxidative stress genes (*ahpC* and *tpx*) was lower in the  $\Delta luxS$  mutant than that in the wt when the cells were treated with  $H_2O_2$ , supporting the observed higher sensitivity of the  $\Delta luxS$  mutant to oxidative stress.

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