The role of Autoinducer 2 in *Campylobacter jejuni*

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Background

Numerous bacteria regulate physiological functions in a cell-dependent manner by communication with each other (Quorum sensing). This process specifically targets and affects bacterial growth and viability as well as the production of virulence factors and is mediated via the small interspecies-specific signaling molecule autoinducer-2 (AI-2). AI-2 is generated as a by-product via LuxS throughout the methionine cycle. The role of AI-2 mediated Quorum sensing in *Campylobacter* (*C.*) *jejuni* is discussed controversially as no AI-2 receptor has been discovered so far for this species. Several studies of *C. jejuni luxS* mutants showed various results. Additionally, most studies lack complementation of *luxS* mutants with AI-2 and/or a metabolic substance. The sometimes opposing findings might be due to different experimental settings, mutation strategies or background of the strains. Therefor, we examined the impact of strain background, mutation strategy and culture condition on three different *C. jejuni luxS* mutants on growth and motility at 37°C and 42°C. Furthermore we complemented each experiment with synthetic AI-2 or homocysteine as well as both combined.

Material and Methods

Bacterial strains

Campylobacter (*C.*) strains were cultured at 37°C or 42°C in Brucella broth (BB) or on Mueller-Hinton blood agar plates under microaerobic conditions (5% O_2, 10% CO_2). The deletion mutant *C. jejuni* NCTC 11168Δ*luxS* was kindly provided by N. Corcionivoschi (University College Dublin, Ireland), the deletion mutant *C. jejuni* 81-176Δ*luxS* by Y. He (U.S. Department of Agriculture, USA) and the insertion mutant *C. jejuni*81-176::*luxS* by B. Quinones (U.S. Department of Agriculture, USA).

Growth of *C. jejuni*

Growth assays were performed using wildtype (wt) and *luxS* mutants of *C. jejuni* 11168 and *C. jejuni* 81-176 strains. Precultures were inoculated in BB to aprox. 2x 10^5 CFU/ml and incubated under microaerobic conditions at 37°C and 42°C. For complementation assays synthetic AI-2 (OMM Scientific), homocysteine (HC, Sigma Aldrich) or AI-2+HC (10µM or 100µM each) were added to the cultures. Numbers of viable bacteria were determined over 48h by plating serial dilutions of the bacterial suspensions.

Swarming assay

As reduced motility has been observed in *luxS* mutants the swarming ability was assessed at 37°C and 42°C on BB (BBA) or Mueller-Hinton (MHA) swarming plates containing 0.4% agar. For complementation (10µM each) AI-2, HC or AI-2+HC were added to the molten BBA. *C. jejuni* wt and *luxS* mutant were adjusted to 10^8 CFU/ml and 1µl dropped on BBA or MHA. After 24h incubation at 37°C or 42°C the diameters of the swarming halos were measured. Halos of *luxS* mutants were normalized to the wildtype halos (100%).

Results

Growth assay

While the Δ*luxS* mutant of *C. jejuni* 11168 showed significantly reduced cell numbers within mid-exponential (8h) and mid-stationary phase (32h) at both temperatures compared to the wildtype (Fig. 1 A-B), only slight decreases in cell count could be observed for both *luxS* mutants of *C. jejuni* 81-176 at both temperatures compared to the wild type (Fig.1 C-F).

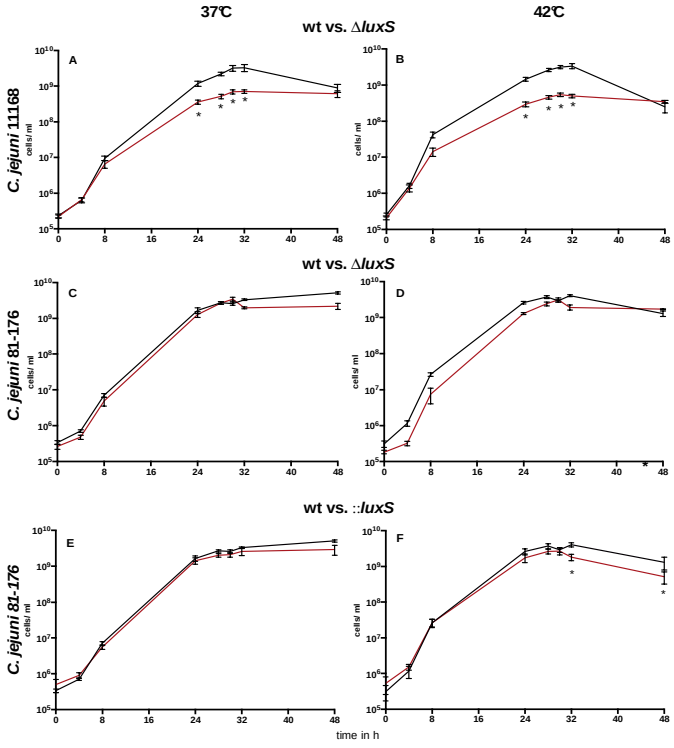

Fig. 1: Growth of *C. jejuni* 11168 and *C. jejuni* 81-176 wt and *luxS* mutants at 37° C and 42° C: A-B) *C. jejuni* 11168 wt/Δ*luxS*, C-D) *C. jejuni* 81176 wt/Δ*luxS*, E-F) *C. jejuni* 81176 wt/::*luxS*; black- wildtype, red- *luxS* mutant; shown are the means ± SD (n=3), *-p<0.05 (Mann-Whitney-U test)

Figure 2 shows the growth curves of *C. jejuni* 11168Δ*luxS* with addition of AI-2 and AI-2+HC combined. Complementation with AI-2 and AI-2+HC significantly increased the cell number of *C. jejuni* 1168Δ*luxS* in stationary phase at 37°C and 42°C compared to the un-complementd Δ*luxS* mutant (Fig. 2A/B). In contrast HC alone did not show any significant effect on cell numbers at any of these temperatures (data not shown). With neither complementation, the cell numbers of the wt-strains was achieved.

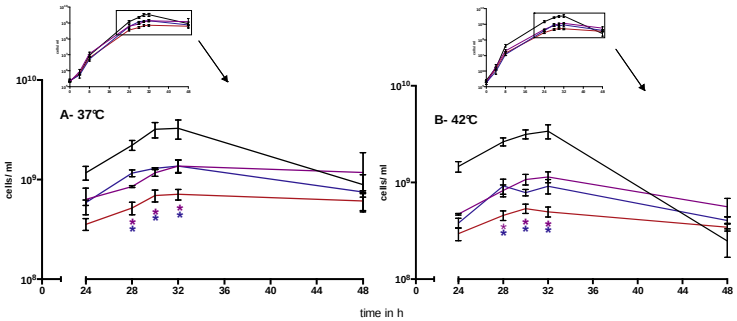

Fig. 2: Growth curves of chemically complemented *C. jejuni* 11168Δ*luxS*: A-37°C, B- 42°C; black- wildtype, red-Δ*luxS*, purple- Δ*luxS*+ AI-2, blue- Δ*luxS*+ AI-2+ HC; shown are mean ± SD (n=5), *- p<0.05 compared to Δ*luxS* (Mann-Whitney-U test)

Swarming assay

Swarming of the *C. jejuni* 11168Δ*luxS* as well as the *C. jejuni* 81-176::*luxS* mutant was significantly lower compared to their corresponding wt at 37°C and 42°C (Fig. 3 A-B). In contrast swarming ability of Δ*luxS* from strain *C. jejuni* 81-176 is not reduced compared to the wt at both temperatures. Complementation showed that the addition of AI-2 to *C. jejuni* 11168Δ*luxS* mutant contribute to an increased swarming ability compared to the uncomplemented mutant at 37°C but not at 42°C. Neither the addition of HC alone nor AI-2 +HC increased swarming ability at both temperatures. The addition of both AI-2+HC to *C. jejuni*81-176::*luxS* mutant yields an increased swarming motility at 37°C, while the swarming ability of *C. jejuni* 81-176Δ*luxS* was not significantly changed by any condition investigated.

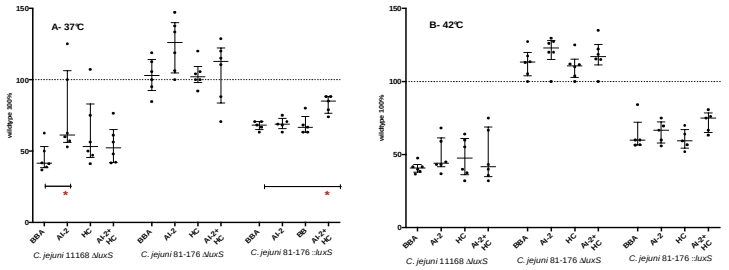

Fig.3: Swarming ability of chemically complemented *C. jejuni luxS* mutants: A-37°C, B- 42°C, complementation with: AI-2, HC, AI-2+ HC, shown are the normalized median and interquartile range (n= 6), *-p<0.05 (Mann-Whitney-U test)

Figure 4 shows the swarming ability of *C. jejuni luxS* mutants on MHA and BBA media. *C. jejuni* 81-176Δ*luxS* exhibits significant reduced swarming on MHA compared to swarming on BBA at both temperatures. The swarming ability of *C. jejuni* 81-176::*luxS* slightly increased on MHA compared to BBA at 42°C but nevertheless no difference could be observed at 37°C. Likewise the swarming ability of *C. jejuni* 11168Δ*luxS* did not differ between MHA and BBA at both temperatures.

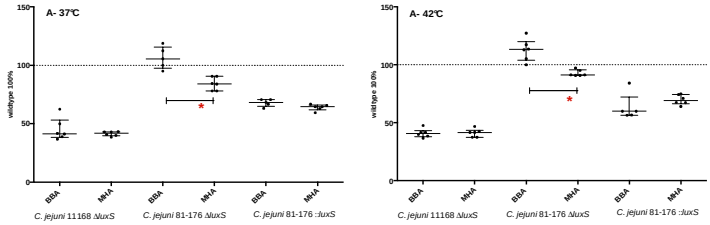

Fig.4: Swarming ability of *C. jejuni luxS* mutants on different media: A-37°C, B- 42°C; shown are the normalized median with interquartile range, *-p< 0.05, (Mann-Whitney-U test); calculation of significance between: BBA vs. MHA

Discussion

Our analyses demonstrated, that occurring phenotypes of *C. jejuni luxS* mutants are depending on strain background, kind of mutation and experimental conditions.

Further, our data implicate that altered phenotypes of *luxS* mutants not solely occur as a consequence of lacking AI-2, as the addition of AI-2 to the *C. jejuni* 11168Δ*luxS* mutant did not completely restore wt-levels during growth and swarming assays (Fig.2 and 3). Adding HC to the mutant strain did not alter the *luxS* mutant phenotype suggesting that disrupting the methionine cycle downstream of LuxS is not responsible for the observed phenotypes. The accumulation of components upstream of LuxS within the methionine cycle as well as other unknown functions of LuxS or mutational downstream effects could also have an impact on the observed phenotypes. Sufficient complementations are required to get an idea how *luxS* is involved in the resulting phenotypes.

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