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The signalling molecule Autoinducer-2 is not internalised in *Campylobacter jejuni*

Das Signalmolekül Autoinducer-2 wird in Campylobacter jejuni nicht internalisiert

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Summary

Bacteria recognise and constantly adjust to changing situations by sensing environmental and self-produced signals. Autoinducer-2 (AI-2) is a signal molecule, found in many bacterial species and thus proposed to enable interspecies communication. Two classes of AI-2 receptors have been identified so far. One class of AI-2 receptors sense AI-2 by a two component signalling system. The other class, the LsrB family, internalises and phosphorylates AI-2. *Campylobacter* (*C.*) *jejuni* is known to produce AI-2, but no AI-2 receptor in *C. jejuni* has been found yet. Therefore, all research on AI-2 dependent phenotypes has been conducted with AI-2 synthase (*luxS*) mutants. This mutation also leads to a disruption of the activated methyl cycle. Most studies lack sufficient complementation resulting in not knowing whether phenotypes of *luxS* mutants depend on disrupted metabolism or lack of AI-2. All this contributes to an intensive discussion about the exact role of AI-2 in *C. jejuni*. Our previous study showed altering phenotypes (growth and motility) of the *C. jejuni* NCTC 11168Δ*luxS* mutant in contrast to wild type, which could be complemented with synthetic AI-2. To assess how AI-2 induces the altering phenotypes during complementation assays, we performed an AI-2 uptake assay. Our data show, that AI-2 is not internalised by *C. jejuni*. These data suggest, that yet unknown AI-2 receptors are present on the cell surface of *C. jejuni*. Therefore further search of AI-2 receptors in *C. jejuni* should focus on two component signalling systems and not on transporter systems.

Keywords: *Campylobacter jejuni*, Autoinducer-2, internalisation

Zusammenfassung

Bakterien sind in der Lage, verändernde Umweltbedingungen zu erkennen und sich diesen anzupassen, indem sie äußere Umwelteinflüsse und selbst produzierte Signale wahrnehmen. Autoinducer-2 (AI-2) ist ein Signalmolekül, das von vielen Bakterienspezies synthetisiert wird. Daher wird vermutet, dass AI-2 der interspezies-spezifischen Kommunikation dient. Bisher sind zwei Klassen von AI-2 Rezeptoren eindeutig identifiziert. Eine Klasse von AI-2 Rezeptoren bindet AI-2 über ein Zwei-Komponenten Signalsystem. Die andere Klasse (LsrB) internalisiert und phosphoryliert AI-2. *Campylobacter (C.) jejuni* synthetisiert AI-2, allerdings konnte bislang noch kein Rezeptor in *C. jejuni* identifiziert werden. Daher wurden sämtliche Studien von AI-2-abhängigen Phänotypen mit AI-2-Synthase (*luxS*)-Mutanten durchgeführt. Allerdings spielt LuxS auch eine zentrale Rolle bei der Regeneration von Homocystein im Methioninzyklus. In vielen Studien fehlt dabei eine ausreichende Komplementation, um zu zeigen, ob auftretende Phänotypen ein Resultat der Störung der metabolischen Funktion von LuxS sind oder sich diese als Konsequenz der Unterbrechung der AI-2 vermittelten Zellkommunikation zeigen. Eine frühere Studie zeigte, dass unterschiedliche Phänotypen von *C. jejuni* NCTC 11168Δ*luxS* Mutanten (Wachstum und Schwärmverhalten) im Vergleich zum Wildtyp auftreten. Diese Unterschiede konnten teilweise mit synthetischem AI-2 komplementiert werden. Um zu untersuchen, wie AI-2 diese

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veränderten Phänotypen während der Komplementation induziert, wurde hier ein AI-2 uptake assay durchgeführt. Dabei zeigte sich, dass AI-2 nicht aktiv in *C. jejuni* internalisiert wird. Unsere Daten lassen vermuten, dass noch unbekannte AI-2 Rezeptoren in *C. jejuni* vorhanden sind. Somit sollte sich die weitere Suche nach AI-2 Rezeptoren in *C. jejuni* auf Zwei-Komponenten-Systeme konzentrieren und nicht auf Transportersysteme.

Schlüsselwörter: *Campylobacter jejuni*, Autoinducer 2, Internalisierung

Introduction

Many bacterial species recognise and regulate their behavior through an intercellular signalling system by sensing environmental and self-produced signals. This process is commonly known as Quorum sensing (QS), which allows bacteria to communicate with each other and therefore coordinate their activities at a multicellular level. QS has been demonstrated to play a significant role in a variety of bacterial processes, including motility, biofilm formation, expression of virulence genes, and bioluminescence (Engebrecht et al., 1983; Davies et al., 1998; Miller et al., 2002). One QS signal molecule is the Autoinducer 2 (AI-2), generated via LuxS (Bassler et al., 1999), an enzyme found in many bacterial species and thus expected to enable interspecies communication.

LuxS has been found in many different bacteria taxa, even though an AI-2 receptor has not been detected in all bacteria, leading to the question whether the presence of *luxS* is always correlated to an AI-2 dependent QS regulated behaviour (Rezzonico and Duffy, 2008). On the other hand it has been shown that *Sinorhizobium meliloti* respond to AI-2 even though it lacks a *luxS* gene suggesting different AI-2 mediated QS mechanisms (Pereira et al., 2008).

Despite the large number of studies identifying AI-2 regulated phenotypes, the mechanisms of AI-2 detection has only been determined in some bacterial species. Three classes of AI-2 receptors, involved in downstream signalling, have been identified so far. In many bacteria e. g. *Salmonella* spp. and *Escherichia (E.) coli*, AI-2 binds to LsrB, the ligand binding protein of an ABC transporter, which is exposed at the cell surface (Taga et al., 2001; Xavier and Bassler, 2005). During internalization, AI-2 is phosphorylated by the kinase, LsrK, to form phospho-AI-2. Phosphorylated AI-2 binds the transcriptional repressor LsrR so that LsrR is inactivated and the *lsr* operon could be transcribed (Fig. 1A) (Taga et al., 2003). This class of AI-2 receptors are prevalent mainly in *Enterobacteriaceae, Pasteurellaceae, Rhizobiaceae* and *Bacillaceae*, but were not detected in *Vibrionaceae* (Taga et al., 2001; Xavier and Bassler, 2005; Rezzonico and Duffy, 2008; Pereira et al., 2009). The AI-2 receptor in *Vibrio* spp., is the periplasmic two component sensor kinase LuxPQ (Reading and Sperandio, 2006). Here, just the signal but not the AI-2 molecule is transduced inside the cell (Fig. 1B). In *Vibrio (V.) harveyi*, AI-2 binds to the LuxP receptor protein, thereby inducing a phosphorylation-dependent signalling cascade of LuxQ, LuxU and LuxO. Dephosphorylated LuxO enhances protein synthesis of the transcriptional activator LuxR, which results in increased expression of the *lux*-operon (Freeman and Bassler, 1999a; Freeman and Bassler, 1999b). This class of AI-2 receptor has been identified only in *Vibrio* spp. (Rezzonico and Duffy, 2008). Recently, chemoreceptors in *E. coli* and *Helicobacter (H.) pylori* have been described as third class of AI-2 receptors (Hedge et al., 2011; Rader et al., 2011). AI-2 is perceived as chemoattractant in *E. coli* via the chemoreceptor Tsr and LsrB (Hegde et al., 2011), while Rader et al. (2011) identified AI-2 as chemorepellent in *H. pylori* sensed by the chemoreceptor TlpB. The signal transduction mechanism of AI-2 via TlpB is not known so far. Also it is not clear whether TlpB senses AI-2 directly or via other binding proteins (Rader et al., 2011).

The veterinary importance of *C. jejuni* infection is primarily the contamination of carcasses and based on this the risk of foodborne disease. There is an essential need for an improved understanding of the pathogenic mechanisms responsible for the disease manifestations of this organism. The existence of LuxS, as well as the LuxS-dependent AI-2 production in *C. jejuni* NCTC 11168, was first described by Elvers and Park (2002). As no AI-2 receptor has been identified for *C. jejuni* so far, several studies investigated AI-2 dependent phenotypes by using *C. jejuni luxS* mutants. Phenotypes of *C. jejuni luxS* mutants were controversially described and are summarised in some review articles (Golz et al., 2012; Plummer, 2012). Since LuxS displays a primary role as metabolic enzyme in the activated methyl cycle (AMC), phenotypes of *luxS* mutants could depend on lack of AI-2 signalling or disrupted AMC. The fact that AI-2 is a by-product of the AMC and that a receptor is yet to be found, leads to the question, if AI-2 in *C. jejuni* is indeed a true QS signal molecule. As previously reported the Δ*luxS* mutant of *C. jejuni* NCTC 11168 showed significantly reduced cell numbers compared to the wild type and reduction of swarming ability (Adler et al., 2014). Since the *C. jejuni* NCTC 11168Δ*luxS* phenotypes could be partially complemented with synthetic AI-2, an AI-2 mediated signalling mechanism via AI-2 receptors seems possible. However, no homologues of the known AI-2 receptors like LuxP or LsrB were identified in *Campylobacter* spp. (Cloak et al., 2002; Rezzonico and Duffy, 2008). Despite of the existence of chemoreceptors in *C. jejuni*, no TlpB or Tsr receptor homologous has been found yet (Golz et al., 2012).

The recognition of AI-2 in *C. jejuni* could be achieved by binding of AI-2 to a periplasmic protein like in *V. harveyi* and *H. pylori* or by an uptake of AI-2 molecules like in *E. coli*. Here we investigate if AI-2 molecules were imported through an ABC Transporter system by *C. jejuni* via an AI-2 uptake assay.

Material and Methods

Bacterial strains and growth conditions

Campylobacter strains described in Table 1 were cultured at 37°C in Brucella broth (BB) (BD, Heidelberg, Germany)

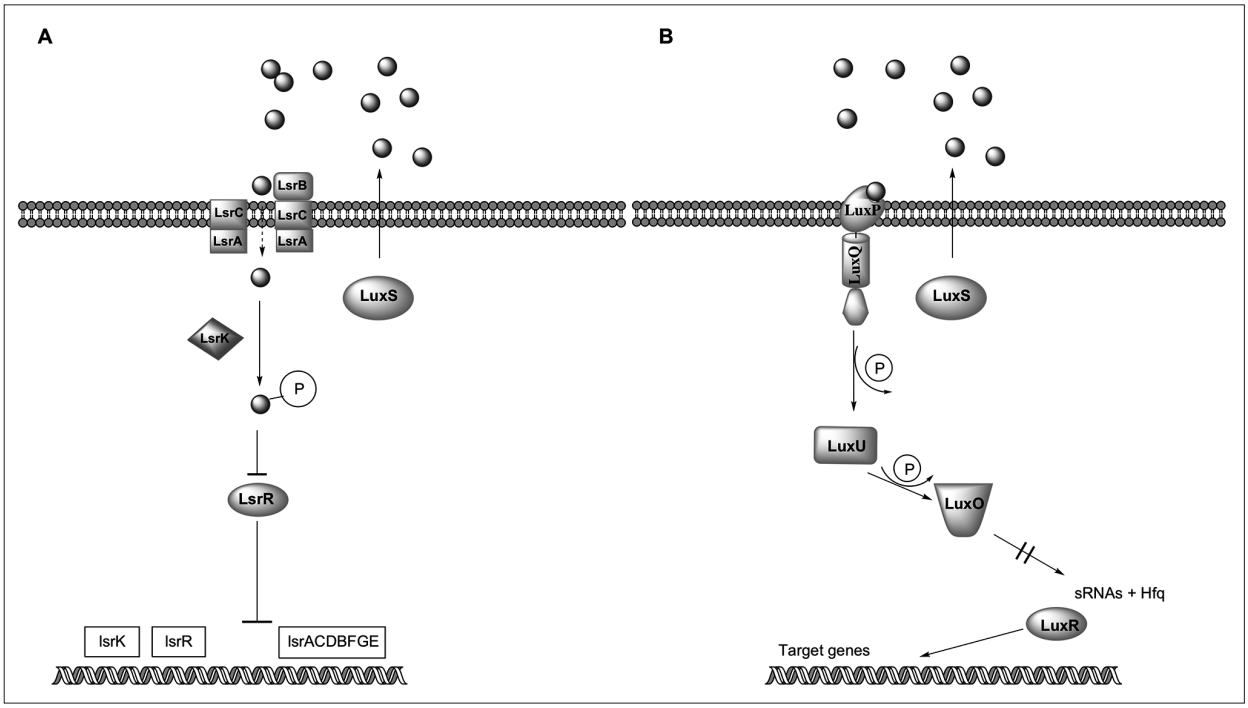

FIGURE 1: *Quorum sensing system of E. coli (A) and V. harveyi (B): In V. harveyi, AI-2 binds to the LuxP receptor protein, thereby inducing a phosphorylation-dependent signalling cascade of LuxQ, LuxU and LuxO. Dephosphorylated LuxO enhances protein synthesis of the transcriptional activator LuxR, which results in increased expression of the lux-operon. The import of AI-2 by the ABC-transporter (composed of LsrA, LsrB and LsrC) in E. coli results in phosphorylation of the signal molecules by LsrK. Phosphorylated AI-2 inactivates LsrR (transcriptional repressor) and thereby increases the expression of the lsr-operon and can modulate the transcription of other target genes.*

or on Mueller-Hinton blood agar plates (MHB) (Oxoid, Wesel, Germany) under microaerobic conditions (5% O_2, 10% CO_2) generated by an Anoxomat (Omni Life Science, Bremen, Germany). *V. harveyi* strains were cultured at 30°C under aerobic conditions on Luria Bertani (LB) agar plates (BD, Heidelberg, Germany) or in Autoinducer Bioassay (AB) media (Greenberg et al., 1979). *E. coli* was cultured at 37°C under aerobic conditions in BB or on LB agar plates. The mutation of *luxS* was confirmed by sequencing the *luxS* gene and by the absence of AI-2 activity in the *V. harveyi* bioluminescence assay.

Quantification of AI-2

AI-2 activity of the synthetic AI-2 (OMM Scientific, Dallas, USA) was quantified with the bioluminescence assay and compared to wild-type *C. jejuni* grown to an OD600 nm of 1, at which maximal AI-2 activity was obtained for *C. jejuni* NCTC 11168. Synthetic AI-2 activity of a concentration of 10 µM was comparable with AI-2 activity achieved with the *C. jejuni* wild type strain. Accordingly, for AI-2 uptake assay AI-2 at a concentration of 10 µM and non-limiting concentration of 100 µM was used.

AI-2 uptake assay

Overnight cultures of the AI-2-deficient mutant *C. jejuni* NCTC 11168Δ*luxS*, *E. coli* and *V. harveyi* (BB170) were diluted in BB or AB to a cell density of $1x10^8$ CFU/ml. After cell adjustment, AI-2 (10 µM and 100 µM) was added. As positive controls AI-2 (10 µM and 100 µM) alone, and the particular bacteria strain (excluded *C. jejuni* NCTC 11168Δ*luxS*, no AI-2 production) was tested. To determine the amount of extracellular AI-2 at each time point (0 h, 2 h, 4 h, 6 h), culture supernatants were collected. Cell free supernatants (CFS) were achieved by centrifugation at 8000x g for 10 min and sterilization by passing through a 0.2 µm filter (VWR, Darmstadt, Germany) and stored at –20°C until used. In parallel the absorbance was measured at the same time point to determine cell growth.

TABLE 1: *Bacterial strains used in this study*

Strains	*luxS*	Description	Source or reference
E. coli K12 DH5α	-	AI-2 uptake	ATCC 23716
V. harveyi BB152	+	AI-2 production, positive control	ATCC BAA-1119
V. harveyi BB170	+	Reporter strain for AI-2, AI-2 receptor binding	ATCC BAA-1117
C. jejuni NCTC 11168	+	Wildtype, Isolated from clinical sample in the UK in 1977	NCTC 11168
C. jejuni NCTC 11168Δ*luxS*	-	*luxS*-deletion mutant, kanamycin resistance cassette	Corcionivoschi et al., 2009

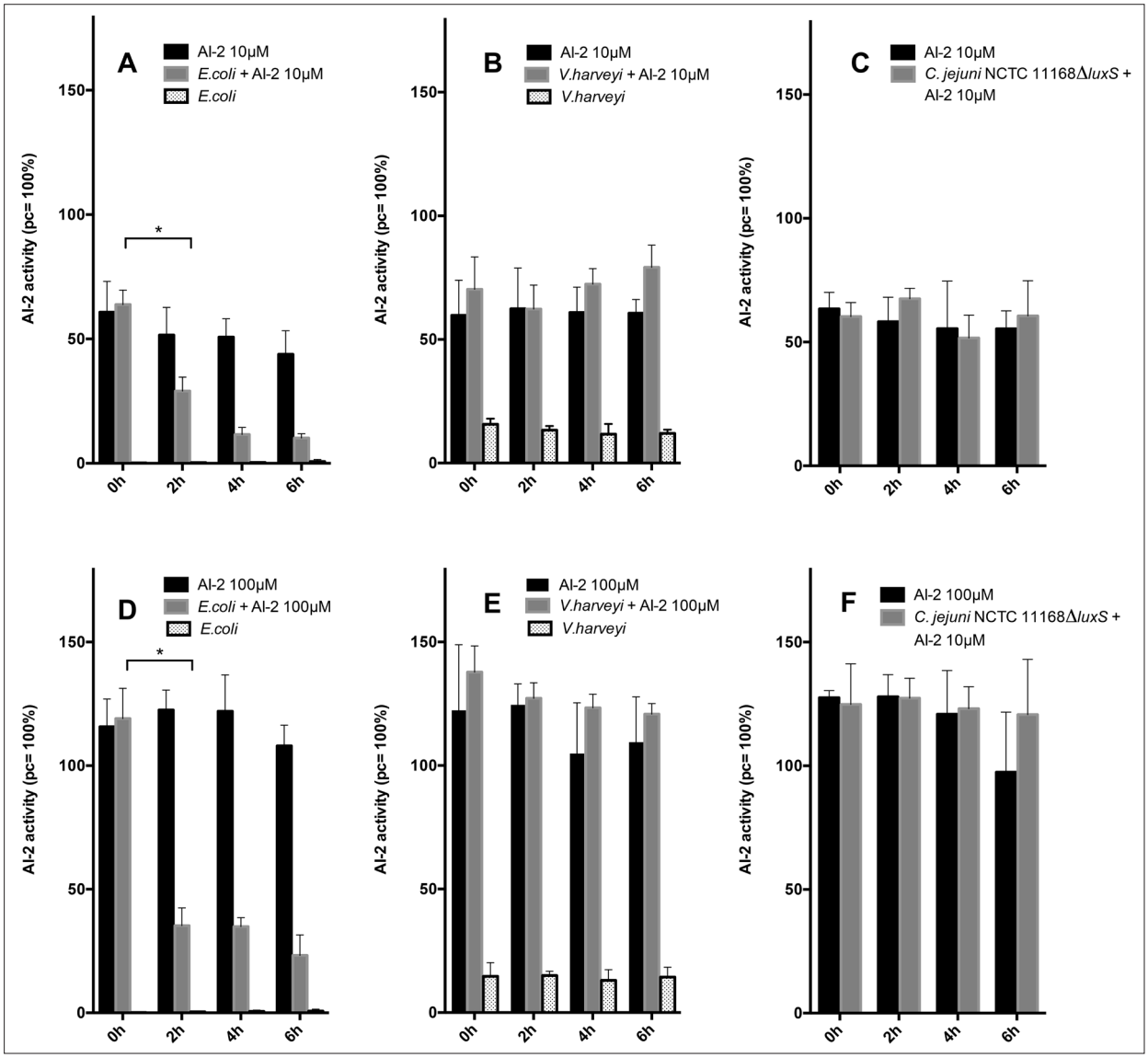

FIGURE 2: *AI-2 uptake assay of E. coli (A/D), V. harveyi (B/E) and C. jejuni NCTC 11168ΔluxS (C/F): Strains were grown in AB/BB medium to a cell density of 1x108 CFU/ml, and AI-2 (10 µM respectively 100µM) was added. Supernatants were collected, and the V. harveyi autoinducer assay was performed to determine the amount of extracellular AI-2 remaining. Three independent cultures were used to generate the AI-2 uptake profile; * –p < 0.05 (Mann-Whitney-U test); pc- positive control.*

The *V. harveyi* bioluminescence assay was performed as described previously (Surette and Bassler 1998). Strain *V. harveyi* BB152 functioned as positive control. The *V. harveyi* reporter strains (BB170) were grown over night in AB medium and diluted (1:5000) into fresh AB medium. CFS and uninoculated AB respectively BB medium were then added to the diluted *V. harveyi* culture at 10% (v/v) final concentration. Reporter strains with CFS or sterile media were incubated at 30°C with aeration (750 rpm). After 4 hours of incubation, luminescence of 100 µl aliquots in microtiter plates were measured (10 s per well) using Luminometer (CentroPro, Berthold, Bad Wildbach, Germany). For each of three experiments, triplicates of relative light units (RLU) were measured. n-fold luminescence induction values were calculated from RLU obtained with conditioned CFS vs. RLU obtained with sterile medium.

Statistical analysis

Experiments were repeated three times in three independent experiments. Statistical analyses were performed using GraphPad Prism v6.0 (GraphPad Prism, San Diego, USA). To calculate significant differences a two-tailed Mann-Whitney test was used with a confidence level of 95%.

Results

To investigate how AI-2 is recognised by *C. jejuni* NCTC 11168Δ*luxS*, we conducted an AI-2 uptake assay. To ensure that the AI-2 uptake assay is able to recognise if exogenous AI-2 was taken up by the cells, we included an *E. coli* strain, which is known to express an ABC transporter system for AI-2 uptake. *V. harveyi* itself induces luminescence in the bioluminescence reporter assay at around 15% compared to the positive control (Fig. 1B),

whereas *E. coli* itself induces luminescence only at a low level of 1.5% (Fig. 1A). *C. jejuni* NCTC 11168Δ*luxS* alone is not able to produce AI-2, hence we abstained to investigate the ability of luminescence induction of this strain alone. Nevertheless, routine measurement of AI-2 activity from this strain revealed that no luminescence induction takes place. Synthetic AI-2, at a concentration of 10 µM, induces an AI-2 activity of approximately 55% compared to the positive control, whereas a concentration of 100 µM AI-2 induces an activity of 120% in the *V. harveyi* bioluminescence reporter assay. After 2 h of incubation the AI-2 activity in the supernatants of *E. coli* cultures significantly decreased, thereby approximately 54% of the exogenous AI-2 (10 µM) was absorbed by *E. coli* (Fig. 2A/D) and AI-2 activity declined further to 15% after 6 h. Respectively, only 30% of the exogenous AI-2 activity (100 µM) was measured after 2 h, declining to 21% after 6 h. The disappearance of AI-2 from the medium suggests that exogenous AI-2 was taken up by *E. coli* cells. The AI-2 receptor binding of *V. harveyi* did not diminish AI-2 activity in the supernatant in either concentration of exogenous added AI-2 (10 µM and 100 µM) within the investigated time period of 6 h (Fig. 2B/E). The AI-2 activity remained around 70% at an AI-2 concentration of 10 µM, and around 120% at an AI-2 concentration of 100 µM. Also, no disappearance of AI-2 activity in the supernatants of *C. jejuni* NCTC 11168Δ*luxS* cultures with 10 µM or 100 µM AI-2 addition was evident at any time point investigated here (Fig. 2C/F). These data suggest that AI-2 was not actively taken up by *C. jejuni* NCTC 11168Δ*luxS*.

Discussion

Since the discovery that *C. jejuni* produce AI-2, various studies have been conducted to explore the function and role of AI-2 in *C. jejuni* (Golz et al., 2012; Plummer, 2012).

Adhesion to the intestine epithelia and invasion into the cells is vital for the pathogenicity of *Campylobacter* spp. Plummer et al. (2012) recently showed that one *C. jejuni* strain (IA3902) completely lost its ability to colonize the intestinal tract of guinea pigs and chicken, when QS is inhibited (*luxS* knock-out). Another strategy to inhibit QS could be the occupation of appropriated QS receptors. Therefore we try to understand which kind of QS receptors exist in *C. jejuni.* No orthologs of the described AI-2 recptors have been detected in *C. jejuni* and no mechanism of cellular recognition of AI-2 in *Campylobacter* is known so far. Mutation of the AI-2 synthase LuxS also leads to disruption of the AMC, which might also impact the investigated phenotype (Winzer et al., 2002; Rezzonico and Duffy, 2008). Not knowing the AI-2 receptor makes investigations on AI-2 dependent phenotypes much more complicated.

Since some phenotypes of the *C. jejuni* NCTC 11168Δ*luxS* mutant could be complemented with synthetic AI-2 but not with homocysteine (metabolised by LuxS in the AMC) the question arose by which mechanism AI-2 is recognised by *C. jejuni* (Adler et al., 2014). Therefore we examined whether an active uptake of AI-2 takes place in *C. jejuni*.

For the AI-2 uptake assay we used *E. coli* as a positive control, as it has been shown that *E. coli* import AI-2 through the Lsr ABC transporter system (Lee et al., 2008). 10 µM respectively 100 µM synthetic AI-2 was added to *E. coli* cultures and after 2 h the AI-2 activity rapidly decreased from the culture fluids. In contrast, in the supernatants of *V. harveyi* cultures no decrease of AI-2 activity was observed. This is in concordance with the described AI-2 signal transduction mechanism via the two-component signalling system LuxPQ of *V. harveyi* (Reading and Sperandio, 2006). During the AI-2 uptake assay it was shown that also in the supernatants of *C. jejuni* NCTC 11168Δ*luxS* the AI-2 activity did not decrease (Fig. 2C/F), thus it can be concluded that no AI-2 internalization occurs in *C. jejuni* suggesting that the AI-2 signal is transduced by a two component signalling system.

A peculiarity of AI-2 signalling is that the different AI-2 receptors recognise distinct forms of AI-2. For example, *V. harveyi* responds to the borate diester derived from (2S,4S)-THMF, whereas *Salmonella* Typhimurium and *Yersinia pestis* respond to (2R,4S)-THMF (Chen et al., 2002; Kavanaugh et al., 2011; Miller et al., 2004). This demonstrates that the signal molecule ultimately detected by one species can differ. It seems equally probable that the synthetic AI-2 used in this study has an inappropriate structure for recognition in *C. jejuni*. However, in solution, DPD exists as equilibrium of different isomers through cyclization and hydration of DPD (Tsuchikama et al., 2011). Hence, the synthetic AI-2 was able to induce bioluminescence in *V. harveyi* as well as taken up by *E. coli*. This suggests, that the DPD used in this study harboured adequate amounts of both AI-2 variants, and neither of these AI-2 forms is imported by *C. jejuni*.

Since the known AI-2 receptors transduce the AI-2 signal by several distinct mechanisms like import of AI-2 by Lsr ABC-transporter or signal transduction by two component sensor kinases as LuxPQ or chemoreceptors TlpB and Tsr it seems possible that further so far unknown AI-2 receptors exist.

Campylobacter spp. belong to the class of *Epsilonproteobacteria*, representing an ecologically diverse group of microorganisms that are rather evolutionarily distinct from the *Gammaproteobacteria*, such as *E. coli, Salmonella* spp. and *Vibrio* spp. (Gilbreath et al., 2011). This might also explain different kinds of AI-2 perception.

The evolutionary differences as well as the different types of AI-2 recognition systems already described, let us speculate that other kinds of AI-2 receptors exist in *C. jejuni*.

Conclusion

As AI-2 was not actively taken up by *C. jejuni* NCTC 11168Δ*luxS*, future studies on AI-2 reception in *C. jejuni* should rather focus on two-component signalling systems than on ABC-transporters.

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Conflict of interests: The authors declare that they have no conflicts of interest.

References

Adler L, Alter T, Sharbati S, Golz G (2014): Phenotypes of *Campylobacter jejuni luxS* Mutants Are Depending on Strain Background, Kind of Mutation and Experimental Conditions. PLoS One 9: e104399.(Adler et al. 2014)

Bassler BL (1999): How bacteria talk to each other: regulation of gene expression by quorum sensing. Curr Opin Microbiol 2: 582–587.

Chen X, Schauder S, Potier N, Van Dorsselaer A, Pelczer I, Bassler BL, Hughson FM (2002): Structural identification of a bacterial quorum-sensing signal containing boron. Nature 415: 545–549.

Cloak OM, Solow BT, Briggs CE, Chen CY, Fratamico PM (2002): Quorum sensing and production of autoinducer-2 in *Campylobacter* spp., *Escherichia coli* O157:H7, and *Salmonella enterica* serovar Typhimurium in foods. Appl Environ Microbiol 68: 4666–4671.

Corcionivoschi N, Clyne M, Lyons A, Elmi A, Gundogdu O, Wren BW, Dorrell N, Karlyshev AV, Bourke B (2009): *Campylobacter jejuni* cocultured with epithelial cells reduces surface capsular polysaccharide expression. Infection and immunity 77: 1959–1967.

Davies DG, Parsek MR, Pearson JP, Iglewski BH, Costerton JW, Greenberg EP (1998): The involvement of cell-to-cell signals in the development of a bacterial biofilm. Science 280: 295–298.

Elvers KT, Park SF (2002): Quorum sensing in *Campylobacter jejuni*: detection of a *luxS* encoded signalling molecule. Microbiology 148: 1475–1481.

Engebrecht J, Nealson K, Silverman M (1983): Bacterial bioluminescence: isolation and genetic analysis of functions from *Vibrio fischeri*. Cell 32: 773–781.

Freeman JA, Bassler BL (1999a): A genetic analysis of the function of LuxO, a two-component response regulator involved in quorum sensing in *Vibrio harveyi*. Mol Microbiol 31: 665–677.

Freeman JA, Bassler BL (1999b): Sequence and function of LuxU: a two-component phosphorelay protein that regulates quorum sensing in *Vibrio harveyi*. J Bacteriol 181: 899–906.

Gilbreath JJ, Cody WL, Merrell DS, Hendrixson DR (2011): Change is good: variations in common biological mechanisms in the epsilonproteobacterial genera *Campylobacter* and *Helicobacter*. MMBR 75: 84–132.

Golz G, Sharbati S, Backert S, Alter T (2012): Quorum sensing dependent phenotypes and their molecular mechanisms in *Campylobacterales* Eur J Microbiol Immunol (Bp) 2: 50–60.

Greenberg EP, Hastings JW, Ulitzur S (1979): Induction of Luciferase Synthesis in *Beneckea-Harveyi* by Other Marine-Bacteria. Arch Microbiol 120: 87–91.

Hegde M, Englert DL, Schrock S, Cohn WB, Vogt C, Wood TK, Manson MD, Jayaraman A (2011): Chemotaxis to the quorum-sensing signal AI-2 requires the Tsr chemoreceptor and the periplasmic LsrB AI-2-binding protein. J Bacteriol 193: 768–773.

Kavanaugh JS, Gakhar L, Horswill AR (2011): The structure of LsrB from *Yersinia pestis* complexed with autoinducer-2. Acta Crystallogr Sect F Struct Biol Cryst Commun 67: 1501–1505.

Lee J, Zhang X-S, Hegde M, Bentley WE, Jayaraman A, Wood TK (2008): Indole cell signaling occurs primarily at low temperatures in *Escherichia coli*. ISME J 2: 1007–1023.

Miller MB, Skorupski K, Lenz DH, Taylor RK, Bassler BL (2002): Parallel quorum sensing systems converge to regulate virulence in *Vibrio cholerae*. Cell 110: 303–314.

Miller ST, Xavier KB, Campagna SR, Taga ME, Semmelhack MF, Bassler BL, Hughson FM (2004): *Salmonella typhimurium* recognizes a chemically distinct form of the bacterial quorum-sensing signal AI-2. Mol Cell 15: 677–687.

Pereira CS, McAuley JR, Taga ME, Xavier KB, Miller ST (2008): *Sinorhizobium meliloti*, a bacterium lacking the autoinducer-2 (AI-2) synthase, responds to AI-2 supplied by other bacteria. Mol Microbiol 70: 1223–1235.

Pereira CS, de Regt AK, Brito PH, Miller ST, Xavier KB (2009): Identification of functional LsrB-like autoinducer-2 receptors. J Bacteriol 191: 6975–6987.

Plummer PJ (2012): LuxS and quorum-sensing in *Campylobacter*. Front Cell Infect Microbiol 2: 22.

Plummer P, Sahin O, Burrough E, Sippy R, Mou K, Rabenold J, Yaeger M, Zhang Q (2012): Critical role of LuxS in the virulence of *Campylobacter jejuni* in a guinea pig model of abortion. Infect Immun 80: 585–593.

Rader BA, Wreden C, Hicks KG, Sweeney EG, Ottemann KM, Guillemin K (2011): *Helicobacter pylori* perceives the quorum-sensing molecule AI-2 as a chemorepellent via the chemoreceptor TlpB. Microbiology 157: 2445–2455.

Reading NC, Sperandio V (2006): Quorum sensing: the many languages of bacteria. FEMS Microbiol Lett 254: 1–11.

Rezzonico F, Duffy B (2008): Lack of genomic evidence of AI-2 receptors suggests a non-quorum sensing role for *luxS* in most bacteria. BMC Microbiol 8: 154.

Surette MG, Bassler BL (1998): Quorum sensing in *Escherichia coli* and *Salmonella typhimurium*. Proc Natl Acad Sci USA 95: 7046–7050.

Taga ME, Miller ST, Bassler BL (2003): Lsr-mediated transport and processing of AI-2 in *Salmonella typhimurium*. Mol Microbiol 50: 1411–1427.

Taga ME, Semmelhack JL, Bassler BL (2001): The LuxS-dependent autoinducer AI-2 controls the expression of an ABC transporter that functions in AI-2 uptake in *Salmonella typhimurium*. Mol Microbiol 42: 777–793.

Tsuchikama K, Lowery CA, Janda KD (2011): Probing autoinducer-2 based quorum sensing: the biological consequences of molecules unable to traverse equilibrium states. J Org Chem 76: 6981–6989.

Winzer K, Hardie KR, Burgess N, Doherty N, Kirke D, Holden MT, Linforth R, Cornell KA, Taylor AJ, Hill PJ, Williams P (2002): LuxS: its role in central metabolism and the in vitro synthesis of 4-hydroxy-5-methyl-3(2H)-furanone. Microbiology 148: 909–922..

Xavier KB, Bassler BL (2005): Regulation of uptake and processing of the quorum-sensing autoinducer AI-2 in *Escherichia coli*. J Bacteriol 187: 238–248.

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