QUORUM SENSING DEPENDENT PHENOTYPES AND THEIR MOLECULAR MECHANISMS IN *CAMPYLOBACTERALES*

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Quorum sensing comprises the mechanism of communication between numerous bacteria via small signalling molecules, termed autoinducers (AI). Using quorum sensing, bacteria can regulate the expression of multiple genes involved in virulence, toxin production, motility, chemotaxis and biofilm formation, thus contributing to adaptation as well as colonisation. The current understanding of the role of quorum sensing in the lifecycle of *Campylobacterales* is still incomplete. *Campylobacterales* belong to the class of *Epsilonproteobacteria* representing a physiologically and ecologically diverse group of bacteria that are rather distinct from the more commonly studied *Proteobacteria*, such as *Escherichia* and *Salmonella*. This review summarises the recent knowledge on distribution and production of AI molecules, as well as possible quorum sensing dependent regulation in the mostly investigated species within the *Campylobacterales* group: *Campylobacter jejuni* and *Helicobacter pylori*.

Keywords: quorum sensing, LuxS, AI-2, *Campylobacter*, *Helicobacter*

Introduction

Campylobacterales belong to the class of *Epsilonproteobacteria*. This order comprises the two large families, *Campylobacteraceae* and *Helicobacteraceae*. Members of the *Epsilonproteobacteria* represent a physiologically and ecologically diverse group of microorganisms that are rather evolutionarily distinct from the more commonly studied *Proteobacteria*, such as *Escherichia* (*E. coli*) and *Salmonella* spp. [1]. The genera *Campylobacter* (*C.*), *Arcobacter* (*A.*), *Sulfurospirillum* (*Su.*) and *Dehalospirillum* belong to the *Campylobacteraceae*, while the genera *Helicobacter* (*H.*), *Wolinella* (*W.*), *Sulfuricurvum* (*S.*), *Sulfurimonas* (*Sul.*), *Sulfurovum* and *Thiovulum* belong to the *Helicobacteraceae*. The genus *Campylobacter* currently comprises 25 species. Most of them represent commensals of various animals, but particularly two species, *C. jejuni* and *C. coli* are responsible for a large number of acute human gastroenteritis cases worldwide [1]. Approximately 30 species are allocated to the genus *Helicobacter*, which are separated into the gastric and enterohepatic taxa. The most studied *Helicobacter* species is *H. pylori* causing gastritis, gastric ulcer and gastric carcinoma in humans.

Even though autoinducer (AI) mediated quorum sensing has been described for various bacterial species, current understanding of the role of quorum sensing in the

lifecycle of *Campylobacterales* is still incomplete and only limited data are available on the underlying mechanisms. In this review, we summarise and discuss the recent knowledge on quorum sensing in *Campylobacterales*, its molecular mechanisms and the possible functions.

Quorum sensing

The communication of numerous bacteria via small signalling molecules is called quorum sensing. This process directs cell-population and density-dependent regulation of gene expression [2] as crucial processes regulating virulence, toxin production, motility, chemotaxis and biofilm formation, which might contribute to bacterial adaptation and colonisation [3]. Quorum sensing AI signalling molecules mediate intraspecies-specific communication by AI-1 (*N*-acylhomoserine lactones) in Gram-negative bacteria and by oligopeptides in Gram-positive bacteria. AI-2 mediates intra- as well as inter-species specific communication of both Gram-positive and Gram-negative bacteria [2, 3]. A third group of autoinducers, AI-3, was initially described in enterohemorrhagic *E. coli* (EHEC) by Sperandio et al. [4]. This group of molecules mimics eukaryotic hormones and mediates inter-kingdom signalling events among bacteria and mammals or plants and *vice versa* [5]. The function of quorum sens-

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ing mechanisms was first described for bioluminescence production of *Vibrio* (*V.*) *fisheri* and *V. harveyi*. The signalling mechanisms of these autoinducers are highlighted in more detail in other reviews [2, 6–13]. AI-1 signalling has been intensively described in *Vibrio* spp. Briefly, the LuxI-type enzyme produces the AI-1 signalling molecules which can diffuse freely across the bacterial cell wall (Fig. 1). With increasing cell density the level of AI-1 increases. After reaching a critical threshold of AI-1 molecules within the cells, these molecules can bind and thereby stabilise the intracellular receptor LuxR [6]. The LuxR-type/AI-1 complex functions as transcriptional activator of the *luxI*-type as well as the target genes. The AI-2 signalling are best described for *V. harveyi* and *E. coli*: In these systems, the enzyme LuxS cleaves *S*-ribosylhomocysteine to produce homocysteine and 4,5-dihydroxy-2,3-pentanedione (DPD), which is the precursor of AI-2 [14]. DPD undergoes spontaneous cyclisation, hydration and boronate ester formation, thereby forming different derivatives such as *S*-THMF-borate (*V. harveyi*) and *R*-THMF (*E. coli*), which are reported to be AI-2 signalling molecules [6]. These molecules are exported to the environment [15] and after reaching a critical threshold, certain signalling cascades are activated (Fig. 2). In *V. harveyi*, AI-2 binds to the periplasmic binding protein LuxP and thereby induces the phosphatase activity of the associated inner membrane protein LuxQ. This in turn dephosphorylates the two component protein LuxU leading to dephosphorylation of the response-regulator LuxO. Dephosphorylated LuxO stabilises the mRNA of the transcriptional activator *luxR*, resulting in increased production of LuxR and in turn of bioluminescence by *V. harveyi*. In *E. coli*, the extracellular AI-2 molecules bind the LuxP homolog LsrB, which is part of an ABC transporter. As a consequence, AI-2 is imported into the cytoplasm by this transporter where it is phosphorylated by LsrK. Phosphorylated AI-2 binds and inactivates the

Fig. 1. Mechanism of AI-1 mediated signalling in *V. fisheri*. The AI-1 synthase LuxI produces AI-1 signalling molecules which can diffuse across the cell wall. The complex of AI-1 molecules with the intracellular receptor LuxR functions as transcriptional activator of the *lux*-operon resulting in enhanced expression of *luxI* and other genes necessary for production of bioluminescence.

Fig. 2. Mechanism of AI-2 mediated signalling in *V. harveyi* and *E. coli*. The AI-2 signalling molecules are synthesised by the enzyme LuxS and exported to the environment. Two different mechanisms are described for the signal transduction. 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 molecules by the ABC-transporter (composed of LsrA, LsrB and LsrC) in *E. coli* results in phosphorylation of the signalling 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. LsrG, LsrF and LsrE are able to dephosphorylate AI-2.

transcriptional repressor LsrR leading to transcription of the *lsr*-operon. Furthermore, LsrG or LsrF processes phosphorylated AI-2 to a configuration which is unable to inactivate LsrR.

Detection methods of autoinducers

Bassler et al. [16] described different mutants of *V. harveyi* strain BB120 that are either able to sense AI-1 (*V. harveyi* BB886 [17]) or AI-2 (*V. harveyi* BB170 [18]). These mutants are even able to sense AIs produced by different bacterial species. The AI activity is determined by bioluminescence assays, in which reporter strains are incubated with cell free supernatants (CFS) of the suspected bacteria and luminescence production is measured. To sense different kinds of AI-1 molecules, mutants in other species have been constructed by other research groups as summarised by Moorhead and Griffiths [19].

AI-1 in Campylobacteriales

Until today, no genes encoding for AI-1 synthase orthologues neither for *luxI* nor *ainS* have been detected in *C. jejuni* [19]. However, Moorhead and Griffiths [19] were able to show that CFS of the fully sequenced *C. jejuni*

model strain 81-176 and the environmental strain cj11 (isolated from retail chicken) were able to induce bioluminescence in the *V. harveyi* reporter strain BB886, identifying a *N*-(3-hydroxy-butanoyl)-*L*-homoserine lactone (HSL) molecule (3-OH-C4-HSL) produced by *C. jejuni*. This HSL (or HSL mimetic) was termed CjA and increased the transition rate to viable but non-culturable (VBNC) state. CjA was reported to inhibit biofilm formation, affect virulence gene expression and increase IL-8 production of the human intestinal epithelial cell line INT-407 during infection. Furthermore, *C. jejuni* 81-176 was able to respond in different ways to other HSLs, like C4-HSL, C6-HSL, C8-HSL, C10-HSL and C12-HSL. The authors concluded that *C. jejuni* can respond to intrinsic AI-1 molecules as well as to AI-1 molecules produced by the surrounding environment. However, no AI-1 signalling molecules produced or sensed by *Helicobacter* spp. are described so far.

AI-2 in *Campylobacterales*

Metabolic function of *LuxS*

The AI-2 synthase *LuxS* is part of the activated methyl cycle (AMC) enzyme complex, which is important for methionine recycling and methylation of DNA and proteins [20]. Methionine is converted to *S*-adenosylmethionine (SAM) in a reaction catalysed by SAM-synthetase (MetK) (Fig. 3). The SAM-dependent methyltransferase cleaves a methyl residue of SAM thereby producing the toxic prod-

uct *S*-adenosylhomocysteine (SAH). SAH is metabolised to homocysteine by a two step reaction, where the enzyme Pfs (5'-methylthioadenosine/*S*-adenosylhomocysteine nucleoidase) catalyses the cleavage of SAH to adenine and *S*-ribosylhomocysteine (SRH) followed by the *LuxS* catalysed cleavage of SRH to homocysteine and DPD, which is the precursor of AI-2 (as described above) [14]. Eukaryotes, archaeobacteria and some eubacteria are able to metabolise SAH to homocysteine in a one step reaction by the SAH hydrolase [12]. The methyltransferases MetE or MetH then catalyse the conversion of homocysteine to methionine. Most *Helicobacteraceae* genomes encode a complete set of AMC enzymes, while *H. pylori* constitutes an exception [21]. The two known methyltransferases MetE and MetH seem to be absent in *H. pylori*, and *LuxS* is part of a *de novo* cysteine biosynthesis pathway that uses methionine as a reduced sulphur source. BlastP analysis with MetE (CAL35316) and MetH (ZP04809678) has shown their absence in *H. acinonychis*, *H. bizzozeronii*, *H. felis* and *H. suis* (all belonging to the gastric *Helicobacter* taxa [22]), while MetH or MetE appear to be present in enterohepatic *Helicobacter* species (*H. bilis*, *H. canadensis*, *H. cinaedi*, *H. hepaticus*, *H. pullorum* and *H. winghamensis*), as well as in *Sulfurimonas* spp., *S. kuziense* and *W. succinogenes* (Table 1).

LuxS sequence and genomic location

Within 11 *Campylobacter* species, where entirely sequenced and annotated genomes are available, only *C. lari* did not encode *luxS*. The completely sequenced genomes of two *Arcobacter* species as well as *Su. deleyianum* encode *luxS* genes. Within the 12 entirely sequenced and annotated *Helicobacter* species only *H. mustelae* does not encode *luxS*, while *W. succinogenes*, both sequenced *Sulfurimonas* species and *S. kuziense* encode *luxS*. None of the species belonging to the *Campylobacterales* encode the SAH hydrolase. Therefore, the species lacking the *luxS* gene seem to be defective in the methionine recycling or cysteine *de novo* biosynthesis, respectively. For the sequenced *C. lari* strain RM2100 it has been proposed that certain defects in amino acid biosynthetic pathways could be compensated by the large number of encoded peptidases [23]. A comparison of all available *LuxS* sequences of *Campylobacterales* from NCBI database revealed two distinct protein clusters (Fig. 4). One cluster is composed of *H. pylori*, *H. felis*, *H. bizzozeroni*, *H. suis* and *H. acinonychis* with over 90% similarity. These species, belonging to the gastric *Helicobacter* taxa, also showed a distinct cluster in the neighbour-joining tree based on partial *gyrB* sequences [24]. The second *LuxS* cluster is composed of *Arcobacter* spp., *Campylobacter* spp., enterohepatic *Helicobacter* spp. and other species contributing to the order of *Campylobacterales*. Within this second cluster more than 90% sequence similarity was shown for *H. canadensis* with *H. pullorum*, for *H. cinaedi* with *H. hepaticus*, for *C. jejuni* with *C. coli* and *C. upsaliensis*, as well as for

Fig. 3. Metabolic function of *LuxS*. Methionine recycling, methylation of DNA and proteins as well as *de novo* cysteine synthesis is affected by the AMC. The SAM-synthetase MetK converts methionine to *S*-adenosylmethionine (SAM). The cleavage of methyl residues from SAM results in the formation of *S*-adenosylhomocysteine (SAH), which can be converted to homocysteine either in a one- or two-step reaction. The SAH-hydrolase metabolises SAH to adenosine and homocysteine in a one-step reaction. In the other pathway, Pfs cleaves SAH into adenine and *S*-ribosylhomocysteine (SRH), which in turn is cleaved into 4,5-dihydroxyl-2,3-pentanedione (DPD) and homocysteine by *LuxS*. Homocysteine can be converted to methionine by the methyltransferase (MetE or MetH) or to cystathionine and further cysteine by the enzymes CysK and MetB.

Fig. 4. Dendrogram of LuxS and localisation of the *luxS* gene in *Campylobacteriales*. Cluster analysis was done with Bionumerics v6.2 by pairwise alignment and UPGMA. If more than one LuxS sequence was available for a species, the consensus sequence was determined with Bionumerics; otherwise, the strain name is declared. All analysed sequences are listed in Table 1. Numbers indicate the percentage of similarity. Species in blue belong to the family of *Campylobacteraceae* and red to *Helicobacteraceae*. The up- and down-stream located genes of *luxS* in the analysed species are shown on the right-hand side of the dendrogram. Genes not annotated so far are coloured in grey. Gene name or function of the encoded proteins is mentioned within the arrows. *A.* (*Arcobacter*), *C.* (*Campylobacter*), *H.* (*Helicobacter*), *S.* (*Sulfuricurvum*), *Su.* (*Sulfurimonas*), *Sul.* (*Sulfospirillum*), *W.* (*Wolinella*).

C. rectus with *C. showae* and *C. concisus*. All LuxS protein sequences exhibit the invariant His-Xaa-Xaa-Glu-His motif (Fig. 5), which is important in the catalytic activity of LuxS [26] and Gly-92 which is important in LuxS function as shown for *C. jejuni* [27].

The *luxS* genes of sequenced *H. pylori* strains are located within an operon encoding *cysK*, *metB* and *luxS* which are necessary for the *de novo* cysteine biosynthesis pathway [21]. The *luxS* genes of *H. acinonychis*, *H. bizzozeronii*, *H. felis* and *H. suis* are organised in the same operon (Fig. 4). All of these species seem to encode a distinct LuxS sequence as compared to other *Campylobacteriales* members. *In silico* analysis by Doherty et al. [21] suggested that the *cysK-metB-luxS* operon was acquired by a horizontal gene transfer event from a Gram-positive enterococcal bacterium after the separation of these species from other *Helicobacter* species but before their separation from each other. The *luxS* gene of *H. canadensis*, *H. pullorum* and *H. winhamensis* is located between an A/G-specific adenine glycosylase and *ligA* (DNA ligase polydeoxyribonucleotide synthase). Within all analysed *C. jejuni* and *C. coli* strains, the *luxS* gene is located between the *gatB* (aspartyl/glutamyl-tRNA amidotransferase subunit B) and an iron/ascorbate-dependent oxidoreductase. In all other analysed species, *luxS* possesses individual genomic locations.

AI-2 exporter and receptor

The YdgG (TqsA) protein of *E. coli* is described to export AI-2 molecules [15]. This protein belongs to a large group of putative transporters, called the AI-2 exporter superfamily, characterised by a uniform topology composed of eight putative transmembrane segments [15]. BlastP analysis of the YdgG sequence of *E. coli* strain CFT073 (AAN80453.11) versus *Campylobacteriales* in NCBI results in a best match to the hypothetical protein Saut_1581 of *Sulfurimonas autotrophica* DSM 16294 (approx. 40% identities to ADN09628). Further BlastP analysis of Saut_1581 versus *Campylobacteriales* results in good matches for putative transport proteins of *C. fetus*, *C. gracilis*, *C. showae*, *C. hominis*, *A. butzleri* or hypothetical proteins of *A. nitrofigilis*, *C. concisus* and *Sulfurimonas denitrificans*. Whether these proteins function as AI-2 exporter or not has to be elucidated in future studies.

So far no homologues of the LuxP or LsrB have been detected in *Campylobacter* spp. or *Helicobacter* spp. [28]. Armbruster et al. [29] have shown that RbsB is required for AI-2 uptake in *Haemophilus influenzae*. This class of ABC-transporters was also proposed as potential AI-2 receptors by Rezzonico and Duffy [28] because of similarities to the LsrB receptor of *E. coli*. But BlastP analysis

Table 1. Strains analysed in this review

Strain	LuxS accession No	AA	MetE	MetH	MetF	Abbr.
<i>A. butzleri</i> ED-1	BAK69820.1	171	+	+		ABUT
<i>A. butzleri</i> JV22	ZP_07890781.1	171	+	+	+	ABUT
<i>A. butzleri</i> RM4018	YP_001489065.1	171	+	+	+	ABUT
<i>A. nitrofigilis</i> DSM 7299	YP_003654439.1	171	+	+		ANIT
<i>C. coli</i> JV20	ZP_07400938.1	164	+			CCOL
<i>C. coli</i> RM2228	ZP_00367216.1	164	+		+	CCOL
<i>C. concisus</i> 13826	YP_001467850.1	171	+		+	CCON
<i>C. curvus</i> 525.92	YP_001407642.1	171	+			CCUR
<i>C. fetus</i> subsp. <i>fetus</i> 82-40	YP_891321.1	166	+			CFET
<i>C. gracilis</i> RM3268	ZP_05624365.1	167	+			CGRA
<i>C. hominis</i> ATCC BAA-381	YP_001406134.1	164	+			CHOM
<i>C. jejuni</i> RM1221	YP_179319.1	164	+			CJEJ
<i>C. jejuni</i> subsp. <i>doylei</i> 269.97	YP_001397709.1	164	+		+	CJEJ
<i>C. jejuni</i> subsp. <i>jejuni</i> 84-25	ZP_01099262.1	164	+		+	CJEJ
<i>C. jejuni</i> subsp. <i>jejuni</i> 260.94	ZP_01069002.1	164	+		+	CJEJ
<i>C. jejuni</i> subsp. <i>jejuni</i> 327	EFV11286.1	164	+			CJEJ
<i>C. jejuni</i> subsp. <i>jejuni</i> 414	ZP_06372272.1	164	+		+	CJEJ
<i>C. jejuni</i> subsp. <i>jejuni</i> 81-176	YP_001000873.1	164	+		+	CJEJ
<i>C. jejuni</i> subsp. <i>jejuni</i> CF93-6	ZP_01068529.1	164	+		+	CJEJ
<i>C. jejuni</i> subsp. <i>jejuni</i> CG8486	ZP_01809886.1	164	+		+	CJEJ
<i>C. jejuni</i> subsp. <i>jejuni</i> DFVF1099	EFV06602.1	164	+			CJEJ
<i>C. jejuni</i> subsp. <i>jejuni</i> HB93-13	ZP_01070917.1	164	+		+	CJEJ
<i>C. rectus</i> RM3267	ZP_03610776.1	171				CREC
<i>C. showae</i> RM3277	ZP_05364224.1	172	+			CSHO
<i>C. upsaliensis</i> RM3195	ZP_00371160.1	164	+		+	CUPS
<i>H. acinonychis</i> str. Sheeba	YP_665186.1	157				HACI
<i>H. bilis</i> ATCC 43879	ZP_04580164.1	166	+		+	HBIL
<i>H. bizzozeronii</i> CIII-1	YP_004606960.1	152				HBIZ
<i>H. canadensis</i> MIT 98-5491	ZP_04870244.1	160	+	+	+	HCAN
<i>H. cinaedi</i> CCUG 18818	ZP_07805185.1	165	+		+	HCIN
<i>H. felis</i> ATCC 49179	YP_004073387.1	151				HFEL
<i>H. hepaticus</i> ATCC 51449	NP_859707.1	165	+		+	HHEP
<i>H. pullorum</i> MIT 98-5489	ZP_04808703.1	160		+	+	HPUL
<i>H. pylori</i> 26695	NP_206905.2	150				HPYL
<i>H. pylori</i> 51	ACX97326.1	150				HPYL
<i>H. pylori</i> 52	ACX98739.1	150				HPYL
<i>H. pylori</i> 83	AEE69765.1	150				HPYL
<i>H. pylori</i> 908	ADN79246.1	150				HPYL
<i>H. pylori</i> B38	YP_003056931.1	150				HPYL
<i>H. pylori</i> F16	BAJ54715.1	150				HPYL

Table 1. Continued

Strain	LuxS accession No	AA	MetE	MetH	MetF	Abbr.
<i>H. pylori</i> F30	BAJ57282.1	150				HPYL
<i>H. pylori</i> F32	BAJ57699.1	150				HPYL
<i>H. pylori</i> F57	BAJ59204.1	150				HPYL
<i>H. pylori</i> G27	YP_002265732.1	150				HPYL
<i>H. pylori</i> Gambia94/24	ADU80965.1	150				HPYL
<i>H. pylori</i> HPAG1	YP_626846.1	150				HPYL
<i>H. pylori</i> HPKX_438_AG0C1	ZP_03240183.1	150				HPYL
<i>H. pylori</i> HPKX_438_CA4C1	ZP_03243532.1	150				HPYL
<i>H. pylori</i> India7	ADU79359.1	150				HPYL
<i>H. pylori</i> J99	NP_222818.1	150				HPYL
<i>H. pylori</i> Lithuania75	ADU82558.1	150				HPYL
<i>H. pylori</i> P12	YP_002300747.1	150				HPYL
<i>H. pylori</i> PeCan4	YP_003926406.1	150				HPYL
<i>H. pylori</i> Puno120	AEN14756.1	150				HPYL
<i>H. pylori</i> Puno135	AEN17818.1	150				HPYL
<i>H. pylori</i> Shi470	YP_001909597.1	150				HPYL
<i>H. pylori</i> SJM180	YP_003928034.1	150				HPYL
<i>H. pylori</i> SNT49	AEN16317.1	150				HPYL
<i>H. pylori</i> SouthAfrica7	ADU84132.1	150				HPYL
<i>H. pylori</i> v225d	ADI34212.1	150				HPYL
<i>H. suis</i> HS1	ZP_08053653.1	152				HSUI
<i>H. suis</i> HS5	ZP_08054166.1	152				HSUI
<i>H. winhamensis</i> ATCC BAA-430	ZP_04583537.1	152	+	+	+	HWIN
<i>S. kujense</i> DSM 16994	YP_004061184.1	161	+	+	+	SKUJ
<i>Su. autotrophica</i> DSM 16294	YP_003892993.1	171	+	+	+	SAUT
<i>Su. denitrificans</i> DSM 1251	YP_394447.1	167	+	+	+	SDEN
<i>Sul. deleyianum</i> DSM 6946	YP_003304940.1	168	+	+	+	SDEL
<i>W. succinogenes</i> DSM 1740	NP_908224.1	163	+	+	+	WSUC

Strain declaration, accession numbers of LuxS sequence, length of LuxS (AA), existence of different methioninesynthases (MetE, MetH and MetF) as well as abbreviation for the species (Abbr.) are listed in the table and below.

A. (*Arcobacter*), C. (*Campylobacter*), H. (*Helicobacter*), S. (*Sulfuricurvum*), Su. (*Sulfurimonas*), Sul. (*Sulfospirillum*), W. (*Wolinella*).

using its amino acid sequence did not reveal a significant similarity to proteins of the *Campylobacteriales* group. No other AI-2 receptor has been described for *Campylobacter* spp. but recently Rader et al. [30] demonstrated that the chemorepellant receptor TlpB is required in *H. pylori* for AI-2 perception as chemorepellant. The sequence identity between TlpB and *Campylobacteraceae* proteins is less than 40% but it has to be investigated if any of the seven integral membrane chemoreceptors or of the three soluble chemoreceptors described for *C. jejuni* sense AI-2 as chemotactic signal [31].

Function of LuxS and AI-2 activity

AI-2 production

The AI-2-synthase LuxS and AI-2 production in *Campylobacter* was described first for *C. jejuni* NCTC 11168 [32] and until now for 14 other *Campylobacter* spp. [31, 33, 34], but LuxS or AI-2 activity was not detectable in *C. lari*, *C. peloridis* and *C. insulaenigrae* strains [31, 34]. For *C. jejuni* it has been demonstrated that the AI-2 level increases in the supernatant during late exponential growth phase and diminishes during entry into stationary phase

Fig. 5. Alignment of LuxS from *Campylobacterales*. LuxS sequences were aligned by the multiple sequence alignment with hierarchical clustering (Multalin software) [25]. The green boxes indicate the catalytically active site (H-X-X-E-H) and G92 which is important for LuxS activity. The strains are listed in Table 1. Consensus levels: high = 90% are shown in red and low = 50% are shown in blue. Consensus symbols: “!” is anyone of IV, “\$” is anyone of LM, “%” is anyone of FY, “#” is anyone of NDQEBZ.

[33, 35]. AI-2 is produced by *Campylobacter* in different food matrices at various temperatures [33] and expression of *luxS* is up-regulated by cultivation in chicken juice at 5 °C [36]. Furthermore, the expression of *luxS* is negatively regulated by CosR (the campylobacter oxidative stress regulator, cj.0355c) at 42 °C [37]. Within the *Helicobacteraceae* the presence of *luxS* and AI-2 activity was described first for *H. pylori* [38, 39]. Doherty et al. [21] compared LuxS sequences of different *Helicobacter* spp., but no AI-2 assays were performed with species other than *H. pylori*. Maximal AI-2 production in *H. pylori* was measured in the mid-exponential growth phase [38] and activity is diminished when cells enter the stationary phase [39]. The potential function of AI-2 was analysed in different *luxS* mutants; results of these studies are summarised below.

Motility and chemotaxis

Campylobacter and *Helicobacter* species exhibit unusually high velocities and movement in viscous substances compared to many other motile bacteria [40]. While *C. jejuni luxS* mutants showed nearly the same growth rate and motility as wild type strains at 42 °C, a minor reduction of growth rate and decreased motility on soft agar was described at 37 °C [14, 32, 35, 41, 42]. It has been shown that *flaA* but not *flaB* transcription was reduced in a *C. jejuni* 81116 *luxS* mutant, grown for 24 h at 42 °C, although the FlaA and FlaB proteins as well as the total flagellar protein amounts and the flagellar structure seemed to be unaffected [41]. While bacterial motility was not affected at 42 °C, microarray and RT-qPCR analysis of a *C. jejuni* 81-176 *luxS* mutant grown for 17 h at 42 °C showed increased expression profiles for some other flagellar as-

sembly/regulation genes including *flgD*, *flgE*, *fliD*, *fliS*, *flgR*, *flgI*, *flgK*, *flaA*, *flgG2* but not *flaB* or *flhA* [14]. In contrast, microarray analysis of a *C. jejuni* NCTC 11168 *luxS* mutant grown for 8 h at 42 °C performed by Holmes et al. [43] indicated a reduced expression profile of 15 flagellar genes including 12 genes which were shown to be up-regulated in the study by He et al. [14]. Holmes et al. [43] also observed a down-regulation of *flaB* but no regulation of *flhA* expression. These opposing results may be due to the different analysed strains, methods or growth phases investigated. Only Holmes et al. [43] complemented the *luxS* mutant by the addition of exogenously produced AI-2 but observed no restoration in gene expression or motility, and concluded that these effects were due to deletion of *luxS* and not of AI-2 depletion. The *luxS* mutants of the *H. pylori* strains X47-2AL and Alston did not show any obvious defects compared to corresponding wild type strains [39, 44], while motility of other *H. pylori* *luxS* mutants of the strains TK1402, G27, SS1 and J99 was reduced as observed in soft agar motility assays and by microscopy [44–47]. In contrast, no differences in flagella formation were detected using scanning electron microscopy [45, 46]. However, the number and the length of individual flagella were reduced in *H. pylori* J99 *luxS* mutants and these mutants showed reduced transcription of *flaA*, *flgE*, *motA*, *motB*, *flhA* and *fliI* but not *flaB* genes [47]. All described defects affecting motility of *H. pylori* *luxS* mutants were restored by the addition of AI-2 or DPD but not by the addition of cysteine. This confirms that the loss of AI-2 rather than the loss of metabolic or regulatory LuxS functions were responsible for these effects [47]. Rader et al. [46] determined that AI-2 functions as signalling molecule up-stream of the flagellar regulator *flhA* in *H. pylori*. These data suggest that bacterial motility or flagella formation and function in *C. jejuni* is dependent on a functional *luxS* but not AI-2 at 37 °C while in *H. pylori* it is partially under control of AI-2.

Campylobacter and *Helicobacter* species regulate their motility by chemotactic signalling systems, which allow the bacteria to follow favourable chemical gradients in their host environment. For chemotactic signal transduction, four different groups of proteins are necessary: (1) chemoreceptors, (2) core signal proteins, (3) accessory proteins and (4) flagellar switch proteins [40]. Quinones et al. [35] described an enhanced chemoattraction towards amino-acids and reduced chemoattraction towards organic acids for the *C. jejuni* 81-176 *luxS* mutant as compared to the wild type strain. The expression of neither *cheA*, *cheW* (core signal proteins) nor *cheB*, *cheR* and *cheV* (accessory proteins) were differentially regulated in the *C. jejuni* 81-176 *luxS* mutant grown at 42 °C [14]. Whether these gene expression patterns are also seen at 37 °C has to be investigated. However, from these results it was concluded that the described swarming motility defects of the *C. jejuni* 81-176 *luxS* mutant are likely due to be defects in flagellar regulation and not in chemotaxis [14]. Contradictory to He et al. [14], a down-regulation of *cheA* in the *luxS* mutant of *C. jejuni* NCTC 11168 was described by Holmes et al. [43].

Since little is known about AI-2 mediated chemotaxis in *C. jejuni* no conclusion towards possible functions can be drawn at this stage. In *H. pylori*, the chemotactic behaviour to low pH is dependent on the chemoreceptor TlpB [48]. A *luxS* mutant of *H. pylori* strain G27 showed reduced stop frequency in liquid media which was restored by the addition of AI-2 or DPD [30]. As addition of DPD to wild type strain cultures results in a swimming behaviour (as it is described in the presence of the chemorepellant HCl), these authors analysed the chemotactic behaviour of double and single mutants, thereby confirming that AI-2 is perceived as a chemorepellant signal via TlpB.

Biofilm formation

Bacteria occurring environmentally, industrially, or clinically are usually found in biofilms rather than in the planktonic state as often seen under laboratory conditions [49]. Formation of *C. jejuni* biofilms is affected by nutritional and environmental conditions. Mutations in both *flaA/B* or *luxS* genes significantly reduced the ability to form biofilms, while biofilm generation of the *luxS* mutant was increased in the presence of CFS from wild type *C. jejuni*, *Pseudomonas* spp. or *Arcanobacterium pyogenes* [49]. It seems that *H. pylori* favours planktonic growth over biofilm formation in the mucous-rich stomach. The *luxS* mutant strains of *H. pylori* SD14 and SD3 showed two- to threefold increased biofilm formation as compared to their wild type strains [50]. These data show that a *luxS* mutant alters biofilm formation in both *C. jejuni* and *H. pylori* strains but with opposite outcome. While high amounts of AI-2 increases motility in both organisms, it reduces biofilm formation of *H. pylori*, thereby supporting the colonisation of niches with small bacterial numbers, and therefore, may lead to better provision of nutrients whereas in *C. jejuni* it favours biofilm formation in the presence of high AI-2 amounts.

Surface structures

Although relatively little is known about the contribution of capsular polysaccharides (CPS) to the pathogenicity of *C. jejuni*, it has been suggested that CPS plays a role in the infection process [51]. A *luxS* mutant of *C. jejuni* 81116 exhibited a reduced capability of agglutination. Based on the above observation, Jeon et al. [41] speculated that quorum sensing mechanisms are possibly involved in the formation of surface structures. Other authors demonstrated that *C. jejuni* 81-176 alters its surface polysaccharides when co-cultured with epithelial cells, suggesting the existence of a cross-talk mechanism that modulates CPS expression during infection. But this mechanism was not affected in *luxS* or *fliQ* mutants [51]. Cross-talk mechanisms between various bacteria and mammalian host cells have been reviewed by Pacheco and Sperandio [5]. Homologues of such signalling systems, described as AI-3/epi/NE in EHEC, were also found in *Salmonella*, *Shigella*, *Haemophilus* and other bacteria [4, 52]. No effects of AI-2 on the expression of surface structures of *H. pylori* are described so far.

Stress response

The resistance of *C. jejuni* NCTC 11168 *luxS* mutant to oxidative stress was not altered as compared to the wild type strain after incubation at 37 °C [32], while corresponding mutants of *C. jejuni* 81-176 were more sensitive to oxidative stress after incubation at 42 °C [14]. The latter *luxS* mutant showed reduced expression of the stress response genes *aphC*, *tpx* and *groES* after H₂O₂ treatment. The oxidative stress regulator CosR negatively regulates LuxS and the stress response associated proteins SodB, Dps and Rrc, while it positively regulates the stress response protein AhpC in *C. jejuni* NCTC 11168 [37]. Deletion of *cosR* rendered the strain more resistant to oxidative stress. Based on these data it is suggested that LuxS is somehow involved in the oxidative stress response of *C. jejuni*. To our knowledge no data on oxidative stress response of *H. pylori* in combination with LuxS are described in the literature. It has been suggested that *C. jejuni* actually responds and adapts to low temperatures as it still produces ATP, consumes oxygen and responds with changing gene expression at 4 °C, although *C. jejuni* does not encode an orthologue of the cold shock protein CspA [36]. Ligowska et al. [36] described that *C. jejuni* NCTC 11168 survives for a significantly prolonged time in chicken juice at 5 °C as compared to laboratory growth medium. After 30 min cultivation under these conditions one of eight differentially expressed genes was *luxS*. Furthermore, the viability in chicken juice of a *C. jejuni* NCTC 11168 *luxS* mutant significantly decreased as compared to the parental strain at 5 °C. Osaki et al. [45] could show that *H. pylori* TK1402 *luxS* mutants were as resistant to acid stress as its wild type strain, suggesting that this stress response is independent of AI-2 mediated quorum sensing.

Virulence factors and pathogenicity

Putative pathogenicity associated factors of *C. jejuni* include genes for motility and chemotaxis, binding and adhesion, as well as invasion and toxins [53]. The *C. jejuni* NCTC 11168 *luxS* mutant showed no differences in adherence to and invasion of cultured Caco-2 cells as compared to the wild type strain [32]. A *luxS* mutant of *C. jejuni* 81-176 had reduced capabilities in chicken colonisation compared to the wild type strain. This could be due to reduced chemoattraction of this mutant to organic acids which are used for energy generation in the avian gastrointestinal tract. Furthermore, this mutant showed lower adherence to cultured LMH (chicken hepatoma cell line) cells but this effect diminished after genetic complementation of *luxS*, suggesting that LuxS-dependent phenotypes may facilitate the interaction of *C. jejuni* with the chicken host cells [35]. Recently, it has been shown that a *luxS* mutant of the highly virulent sheep abortion *C. jejuni* strain IA3902 completely lost its ability to colonise the intestinal tract of guinea pigs and chicken after oral inoculation, while this *luxS* mutant strain was still virulent after intraperitoneal inoculation of the guinea pigs [42]. Furthermore, these authors showed that a *luxS* mutant of W7 (a motile clone of *C. jejuni* NCTC 11168) showed comparable colonisation

capabilities as the corresponding wild type strain in the chicken model but after co-inoculation of the *luxS* mutant with the wild type strain W7, the mutant was outcompeted by the wild type strain after several days [42]. The phenotypes of genetically complemented *luxS* in the mutant strains of W7 and IA3902 were comparable to the wild type phenotypes.

The cytolethal distending toxin *CDT* is encoded by the three adjacently located genes *cdtA*, *cdtB* and *cdtC* which are part of a polycistronic operon. In *luxS* mutants of *C. jejuni* 81116 *cdt* transcription was reduced compared to wild type and CFS of this mutant induced diminished cell cycle arrest in HeLa cells compared to CFS of the wild type strain [54]. In contrast, Holmes et al. [43] could not confirm a down-regulation of the *cdtA*, *cdtB* and *cdtC* transcripts in a *C. jejuni* NCTC 11168 *luxS* mutant. No obvious changes in the 2D-protein pattern and virulence gene expression between a *luxS* mutant and its isogenic parent *H. pylori* Alston strain *in vitro* were observed by Joyce et al. [39]. The *luxS* mutant of the *H. pylori* strain SS1 showed decreased motility and competitive abilities in wild type co-infected mice, however, the *luxS* mutant of another *H. pylori* strain, X47-2AL, showed none of these defects [44]. Mutation of *luxS* in the *H. pylori* strain TK1402 leads to decreased colonisation of the stomach in infected Mongolian gerbils as well as reduced serum antibody titres against *H. pylori* [45]. Gerbils infected with this mutant exhibited no pathological changes in the stomach *in vivo*, although no differences in adherence to cultured MKN45 cells could be detected between the two strains *in vitro* [45]. These authors mentioned that this *luxS* mutant expressed various virulence factors as they detected unchanged vacuolating cytotoxin activity and elongated phenotype caused by CagA protein injection into epithelial cells through a type IV secretion machinery.

Conclusion

Quorum sensing mediated behaviour often contributes to bacterial survival and pathogenicity. Different aspects of adaptation and virulence regulated by autoinducers in *C. jejuni* and *H. pylori* have been discussed in this review. For *C. jejuni*, the formation of biofilms is AI-2 dependent, but it is not yet clear how AI-2 is involved in this process. All other phenotypes of *C. jejuni* *luxS* mutants were controversially described in the literature. Lacking proofs by genomic complementation of wild type *luxS* or the addition of exogenous AI-2, DPD or CFS produced by wild type strains hamper the interpretation if the obtained phenotypes are a consequence of missing AI-2 molecules or metabolic LuxS function. In addition, some phenotypes, like motility in *C. jejuni* *luxS* mutants, seem to be temperature dependent. Therefore and because of missing data on putative AI-2 exporter and receptors, no clear conclusions can be drawn regarding processes that are regulated by AI-2 quorum sensing mechanisms. However, *C. jejuni* produces and responds to a HSL-mimetic signal molecule

(CjA) with increased transition rate to VBNC state, decreased biofilm formation and altered virulence gene expression. It has been concluded that *C. jejuni* can sense and respond to intrinsic AI-1 molecules as well as those produced by the surrounding microbiota [19]. For *H. pylori* AI-2 mediated quorum sensing mechanisms are described. AI-2 influences flagellar gene expression up-stream of *flhA* and functions as chemorepellant via TlpB. Both mechanisms obviously contribute to regulation of biofilm formation versus planktonic growth which in turn promotes bacterial colonisation and persistence in the stomach [30]. The expression of other virulence factors seems to be regulated by AI-2 independent mechanisms. Interestingly, no AI-1 signalling molecule expression has been identified for *H. pylori* so far. Future studies should clarify how quorum sensing dependent mechanisms function in these species. In addition, two other important questions arise. First, could these AI-mediated mechanisms be used for new therapeutic applications or in reducing the amount of human pathogens in the transmission route by quorum quenching? And second, how do species lacking *luxS* compensate for these regulatory and metabolic mechanisms?

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