10.07.2024

Declaration

I, Jakub Muraszko, hereby declare that my contribution to the following manuscript:

Noszka, M., Strzałka, A., Muraszko, J., Hofreuter, D., Abele, M., Ludwig, C., Stingl, K., Pawlik, A., *CemR atypical response regulator impacts energy conversion in Campylobacteria*. **mSystems** 0:e00784-24 (2024), doi.org/10.1128/msystems.00784-24,

is correctly characterized in the table below.

Contributor	Description of main tasks
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(co-author signature)

11.07.2024

Declaration

I, Dirk Hofreuter, hereby declare that my contribution to the following manuscript:

Noszka, M., Strzałka, A., Muraszko, J., Hofreuter, D., Abele, M., Ludwig, C., Stingl, K., Pawlik, A., *CemR atypical response regulator impacts energy conversion in Campylobacteria.* **mSystems** 0:e00784-24 (2024), doi.org/10.1128/msystems.00784-24,

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Declaration

I, Miriam Abele, hereby declare that my contribution to the following manuscript:

Noszka, M., Strzałka, A., Muraszko, J., Hofreuter, D., Abele, M., Ludwig, C., Stingl, K., Pawlik, A., *CemR atypical response regulator impacts energy conversion in Campylobacteria*. **mSystems** 0:e00784-24 (2024), doi.org/10.1128/msystems.00784-24,

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M. Abela

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10.07.2024

Declaration

I, Christina Ludwig, hereby declare that my contribution to the following manuscript:

Noszka, M., Strzałka, A., Muraszko, J., Hofreuter, D., Abele, M., Ludwig, C., Stingl, K., Pawlik, A., *CemR atypical response regulator impacts energy conversion in Campylobacteria.* **mSystems** 0:e00784-24 (2024), doi.org/10.1128/msystems.00784-24,

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(co-author signature)

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Declaration

I, Kerstin Stingl, hereby declare that my contribution to the following manuscript:

Noszka, M., Strzałka, A., Muraszko, J., Hofreuter, D., Abele, M., Ludwig, C., Stingl, K., Pawlik, A., *CemR atypical response regulator impacts energy conversion in Campylobacteria.* **mSystems** 0:e00784-24 (2024), doi.org/10.1128/msystems.00784-24,

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K. Stingl

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Declaration

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.....Pawlik.....

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3. Complementary research: Cj1608 and Abu0127 ChIP-seq analyses

To complement the data obtained in the work of Noszka et al. (Noszka et al. 2024) (Chapter 2.2) and identify the CjCemR (Cj1608) and AbuCemR (Abu0127) chromosomal binding sites, ChIP-seq analysis was performed for *C. jejuni* NCTC 11168 and *A. butzleri* RM4018, respectively.

3.1. Materials and Methods

3.1.1. Materials and culture conditions

C. jejuni and *A. butzleri* plate cultures were grown on Columbia blood agar base medium supplemented with 5% defibrinated sheep blood (CBA-B). The liquid cultures were prepared in brain heart infusion broth (BHI) (Oxoid) and incubated with 140-rpm orbital shaking. *C. jejuni* and *A. butzleri* cultures were supplemented with antibiotic mixes [*C. jejuni*: vancomycin (5 μg/mL), polymyxin B (2.5 U/mL), trimethoprim (5 μg/mL), and amphotericin B (4 μg/mL) (Contreras et al. 2003); *A. butzleri*: cefoperazone (8 μg/mL), amphotericin (10 μg/mL), and teicoplanin (4 μg/mL) (Atabay and Corry 1998)]. For selecting mutants, appropriate antibiotics were used with the following final concentrations: (i) kanamycin 20 μg/mL (*C. jejuni* and *A. butzleri*) and (ii) chloramphenicol 8 μg/mL (*C. jejuni*). *C. jejuni* and *A. butzleri* were cultivated at 42°C or 30°C, respectively, under optimal microaerobic conditions (*C. jejuni*: 5% O_2, 8% CO_2, 4% H_2, and 83% N_2; *A. butzleri*: 6% O_2, 9% CO_2, and 85% N_2) generated by the jar evacuation-replacement method using Anaerobic Gas System PetriSphere.

3.1.2. Chromatin Immunoprecipitation (ChIP)

Bacterial cultures (70 ml BHI) of *C. jejuni* NTCT 11168 (Parkhill et al. 2000) and *A. butzleri* RM4018 (Miller et al. 2007) wild-types and ΔCj1608 and ΔAbu0127 mutants (Noszka et al. 2024) were grown to OD_{600} of 0.5–0.7. The culture was crosslinked with 1% formaldehyde for 5 min immediately after opening the jar. The crosslinking reactions were stopped by treatment with 125 mM glycine for 10 min at room temperature. The cultures were centrifuged at 4,700 × g for 10 min at 4 °C and washed twice with 25 ml of ice-cold 1 × PBS, followed by the same centrifugation step. Samples were resuspended in 1.1 ml IP buffer (150 mM NaCl, 50 mM Tris-HCl pH 7.5, 5 mM EDTA, 0.5% vol/vol NP-40, 1.0% vol/vol Triton X-100) and sonicated (Ultraschallprozessor UP200s (0.6/50% power, 30 s ON − 30 s

OFF, ice bucket)) to reach 100-500 bp DNA fragment size. Next, the samples were centrifuged at 12,000 × g for 10 min at 4 °C. 100 µl of the supernatant was used for input preparation. 900 µl of the supernatant was incubated with 30 µl of Sepharose Protein A (Rockland, PA50-00-0002) (pre-equilibrated in IP buffer) for 1 h at 4 °C on a rotation wheel. The samples were centrifuged 1000 × g for 2 min at 4 °C; the supernatants were incubated with 100 µl antibody-Sepharose A complex (see chapter 3.1.3) and incubated at 4 °C for 24 h on a rotation wheel. Next, the samples were centrifuged 1000 × g for 2 min at 4 °C, and the supernatant was discarded. The beads were washed four times with IP-wash buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.5% NP-40, 0.1% SDS), twice with TE buffer (10 mM Tris-HCl, pH 8.0; 0.1 mM EDTA), resuspended in 180 µl of TE buffer, and treated with 20 µg/ml RNase A at 37 °C for 30 min. Next, crosslinks were reversed by adding SDS at a final concentration of 0.5% and proteinase K at a final concentration of 20 µg/ml, followed by incubation for 16 h at 37 °C. The beads were removed by centrifugation 1000 × g for 2 min at 4 °C, and the DNA from the supernatants were isolated with ChIP DNA Clean & Concentrator (Zymo Research). The quality of DNA was validated by electrophoresis in 2% agarose gel, and the concentration was determined with QuantiFluor dsDNA System (Promega). The ChIP-DNA was isolated from three independent bacteria cultures.

3.1.3. Preparation of sera

The *C. jejuni* antibody-Sepharose A complex was prepared by adding 40 µg of desalted rabbit polyclonal IgG containing anti-CjCemR antibody to 100 µl of the Sepharose Protein A pre-equilibrated in IP buffer. *A. butzleri* antibody-Sepharose A complex was prepared by adding 120 µg of desalted rabbit polyclonal IgG containing anti-AbuCemR antibody to 100 µl of the Sepharose Protein A pre-equilibrated in IP buffer. The binding reaction was performed on a rotation wheel for 24 h at 4 °C. Next, the complex was washed five times with an IP buffer. The antibodies used in ChIP were raised in rabbits under the approval of the First Local Committee for Experiments with the Use of Laboratory Animals, Wroclaw, Poland (consent number No 053/2020/P2) and validated with Western blot (Figs S11D and S12D in (Noszka et al. 2024)).

3.1.4. ChIP-sequencing

The DNA library preparation and sequencing were performed at the Novogene GmbH (Munich, Germany). Briefly, the DNA fragments were repaired, A-tailed, and further ligated with an Illumina adapter. The final DNA library was obtained by size selection and PCR

amplification. The library was checked with Qubit and RT-qPCR for quantification and a bioanalyser for size distribution detection. The quantified libraries were pooled and sequenced with the NovaSeq X Plus (Illumina), and paired-end 150 bp reads were produced.

3.1.5. ChIP-seq bioinformatic analysis[1]

The 150 bp paired reads were mapped to the *C. jejuni* NCTC 11168 (NC_002163.1) or *A. butzleri* RM4018 (NC_009850.1) genome, depending on the species analysed, using Bowtie2 software with local setting (version 2.5.4) (Langmead and Salzberg 2012; Langmead et al. 2019) and processed using samtools (version 1.20) (Li et al. 2009), achieving more than 10^7 mapped reads on average. Regions differentially bound by Cj1608 or Abu0127 were identified using R packages csaw (version 1.38) with pairend function (Robinson, McCarthy, and Smyth 2010) and edgeR (version 4.2) (Robinson, McCarthy, and Smyth 2010; Lun and Smyth 2016a), following the protocol described in (Lun and Smyth 2016b). Briefly, mapped reads were counted using a sliding window (length 100 bp, slide 33 bp) and filtered using local methods from the edgeR package. Only reads with log fold change (logFC) greater than 1.5 compared to 2000 bp neighbouring region were left for further analysis. Then, each window was tested using the QL F-test, and neighbouring regions (less than 100 bp apart) were merged. The combined p-value of all merged regions was calculated, and only regions with a false discovery rate (FDR) less than 0.05 were considered differentially bound. All identified regions were further confirmed using the MACS3 (version 3.0.1) program with nomodel settings (Zhang et al. 2008) and minimal fold for peaks greater than 1.5. Peaks were detected using mutant strains (ΔCj1608 or ΔAbu0127) and wild-type input DNA as a control. The RNA-seq and ChIP-seq comparison plots were visualised with an R script application created with Shiny (v. 1.8.1) written by Dr Agnieszka Strzałka from the University of Wroclaw (unpublished) with minor modifications.

3.2. Results

3.2.1. Identification of CjCemR (Cj1608) binding sites on *C. jejuni* genome

A ChIP-seq analysis was implemented to identify the CjCemR binding sites on the *C. jejuni* genome. *C. jejuni* wild type (WT) and CjCemR knock-out mutant (ΔCj1608) strains were cultivated in a liquid medium to the logarithmic growth phase (OD_{600} = 0.5) under microaerobic

[1] This part was performed under the supervision of Agnieszka Strzałka, PhD from the University of Wroclaw.

conditions, crosslinked, sonicated, and CjCemR DNA-protein complexes were immunoprecipitated with the polyclonal anti-CjCemR antibody. Parallel control samples were prepared without immunoprecipitation (input, IN). The ChIP DNA was sequenced with a minimum of 3 million reads with mapping above 85% for each sample. Three biological experiments were performed. Two algorithms, MACS3 (more precise for broad peaks) and edgeR (standard usage), were used to identify the binding sites (Fig. 1).

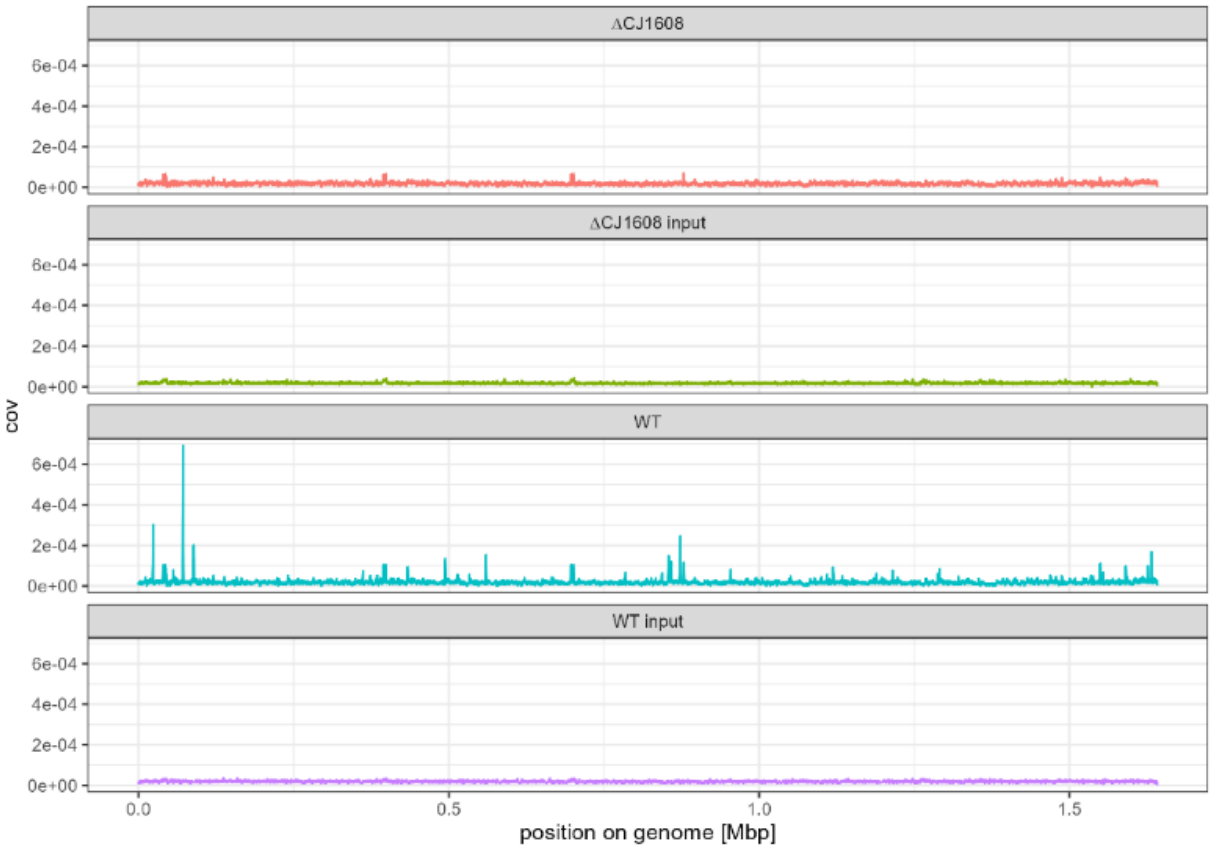

Fig. 1 Identification of CjCemR binding sites on *C. jejuni* NCTC 11168 genome by ChIP-seq. The ChIP-seq reads of Cj1608 immunoprecipitated samples of *C. jejuni* NCTC 11168 wild type (WT) and CjCemR knock-out (ΔCj1608) mutant strains compared with the input DNA samples. COV, read coverage.

We identified 176 binding sites (85 with fold enrichment > 1.5) with MACS3 and 68 with edgeR. Both algorithms identified 60 common binding sites (Tab. 1).

Tab. 1 Identification of CjCemR binding sites on the *C. jejuni* NCTC 11168 genome by ChIP-seq using two algorithms, MACS3 and edgeR. The chromosome position is defined according to the *C. jejuni* NCTC 11168 genome strain (NC_002163.1). FDR, false discovery rate; logFC, log fold change.

ID	ChIP binding start position (bp)	ChIP binding end position (bp)	length of the binding site (bp)	ChIP best position (bp)	P-value	FDR	logFC
1	23233	23926	694	23744	7.26E-10	2.16E-08	4.38
2	55804	56398	595	56348	9.59E-10	2.55E-08	2.64
3	58741	59005	265	58955	2.83E-04	1.80E-03	1.23
4	71479	72172	694	72023	1.18E-09	2.90E-08	5.61
5	88375	89101	727	88820	9.44E-11	6.03E-09	4.06
6	108307	108571	265	108455	4.35E-03	2.27E-02	0.67
7	110122	110584	463	110171	7.05E-05	5.00E-04	0.66
8	118768	119197	430	118817	6.16E-03	3.02E-02	0.60
9	158071	158566	496	158516	2.69E-09	4.77E-08	1.61
10	165430	165958	529	165512	3.94E-08	6.28E-07	1.08
11	226051	226777	727	226100	2.15E-04	1.40E-03	0.80
12	339076	339274	199	339125	5.14E-05	3.81E-04	0.98
13	361318	361912	595	361664	7.46E-10	2.16E-08	2.64
14	364684	364783	100	364733	8.04E-03	3.83E-02	0.35
15	384088	384616	529	384566	6.05E-03	3.02E-02	1.05
16	416791	417088	298	416840	1.17E-06	1.49E-05	1.36
17	433753	434347	595	433802	1.76E-05	1.34E-04	1.83
18	489622	489985	364	489836	4.16E-03	2.25E-02	0.64
19	494011	494704	694	494060	2.20E-09	4.39E-08	2.49
20	513118	513481	364	513431	1.01E-04	6.97E-04	1.40
21	533083	533710	628	533396	5.66E-04	3.41E-03	1.21
22	559483	560143	661	559961	5.19E-10	1.84E-08	3.43
23	588424	589150	727	588473	6.31E-05	4.57E-04	0.63
24	650893	651355	463	650942	2.12E-07	3.08E-06	1.08
25	668482	669010	529	668729	1.08E-05	9.08E-05	1.29
26	784411	784939	529	784757	2.06E-09	4.39E-08	2.63
27	825892	826354	463	825941	1.11E-03	6.56E-03	0.97
28	833581	834010	430	833960	2.81E-06	2.49E-05	1.27
29	843382	843613	232	843563	1.51E-06	1.59E-05	1.67
30	854140	854701	562	854585	2.75E-10	1.10E-08	3.21
31	858298	858892	595	858842	1.30E-12	2.07E-10	3.57
32	872620	873313	694	873230	1.52E-09	3.47E-08	3.42
33	877933	878857	925	878807	8.50E-03	3.99E-02	1.29
34	901099	901594	496	901544	2.69E-06	2.49E-05	0.76

35	931294	931459	166	931343	4.30E-03	2.27E-02	0.58
36	943537	943801	265	943586	4.37E-04	2.72E-03	0.68
37	953833	954460	628	954014	6.73E-07	8.95E-06	1.91
38	1004983	1005181	199	1005131	1.61E-06	1.61E-05	1.28
39	1027786	1027951	166	1027901	3.66E-03	2.05E-02	0.49
40	1050721	1051216	496	1051034	2.58E-06	2.49E-05	1.47
41	1109395	1109923	529	1109675	1.50E-06	1.59E-05	1.79
42	1118965	1119295	331	1119212	2.11E-11	1.69E-09	3.05
43	1153945	1154374	430	1154324	2.35E-09	4.40E-08	1.22
44	1188991	1189354	364	1189040	3.68E-07	5.11E-06	2.03
45	1214863	1215457	595	1214978	1.54E-06	1.59E-05	1.85
46	1252285	1252681	397	1252334	1.24E-06	1.53E-05	0.86
47	1270600	1271029	430	1270979	6.10E-03	3.02E-02	0.44
48	1287496	1288090	595	1287941	1.36E-06	1.59E-05	1.89
49	1290598	1290961	364	1290911	5.55E-08	8.42E-07	2.07
50	1320859	1321486	628	1321436	5.25E-12	5.58E-10	1.94
51	1353562	1354090	529	1354040	1.35E-04	9.13E-04	1.06
52	1465663	1466092	430	1466042	9.82E-03	4.54E-02	0.42
53	1534897	1535623	727	1534946	1.52E-06	1.59E-05	1.84
54	1549483	1550110	628	1549565	1.50E-05	1.16E-04	2.01
55	1554004	1554499	496	1554449	2.76E-10	1.10E-08	2.67
56	1564828	1565224	397	1564877	7.70E-03	3.72E-02	0.65
57	1579579	1580008	430	1579958	2.73E-06	2.49E-05	1.00
58	1590271	1590964	694	1590782	1.05E-05	9.01E-05	1.95
59	1626307	1626901	595	1626356	1.74E-04	1.16E-03	1.67
60	1631917	1632577	661	1632131	2.16E-10	1.10E-08	3.79

According to the localisations of the transcription start site (TSS) of *C. jejuni* NCTC 11168 presented by Porcelli et al. (Porcelli et al. 2013), we found that 58 TSS were located between -150 bp and +150 bp from the best binding site position estimated by ChIP-seq analysis (Tab. 2).

Tab. 2 ChIP-seq binding sites location according to transcription start site (TSS). TSS position based on Porcelli et al. (Porcelli et al. 2013). The searching area was determined to be +/-150 bp from the best binding site estimated by ChIP-seq. COG, a cluster of orthologues group; C, energy production and conversion; E, amino acid metabolism and transport; G, carbohydrate metabolism and transport; H, coenzyme metabolism; J, translation, ribosomal structure and biogenesis; M, cell wall/membrane/envelop biogenesis; N, cell motility; NA, not applicable; O, post-translational modification; P, ion transport and metabolism; Q, secondary structure; S, function unknown; T, signal transduction.

ID	TSS (bp)	strand	locus tag	gene	gene product	COG	ChIP best position (bp)
1	56413	-	Cj0037c	NA	cytochrome C	C	56348
2	59049	-	Cj0039c	*typA*	GTP-binding protein TypA	T	58955
3	71935	+	Cj0057	NA	periplasmic protein	-	72023
4	88746	-	Cj0076c	*lctP*	L-lactate permease	C	88820
5	88854	+	asRNA_ Cj0077c	NA	asRNA_Cj0077c	-	88820
6	118705	+	Cj0113	*pal*	peptidoglycan associated lipoprotein	M	118817
7	158367	-	Cj0155c	*rpmE*	50S ribosomal protein L31	J	158516
8	226068	+	Cj0244	*rpmI*	50S ribosomal protein L35	J	226100
9	339034	+	Cj0370	*rpsU*	30S ribosomal protein S21	J	339125
10	384680	-	Cj0418c	NA	hypothetical protein	M	384566
11	416871	-	Cj0450c	*rpmB*	50S ribosomal protein L28	J	416840
12	416953	+	Cj0451	*rep*	ribulose-phosphate 3-epimerase	G	416840
13	433752	+	Cj0469	NA	amino acid ABC transporter ATP-binding protein	E	433802
14	489937	-	Cj0527c	*flgC*	flagellar basal body rod protein FlgC	N	489836
15	494044	+	Cj0531	*icd*	isocitrate dehydrogenase	C	494060
16	513453	-	Cjp11	NA	RNA component of RNase P	-	513431
17	513542	+	Cj0551	*efp*	elongation factor P	J	513431
18	533297	-	Cjp13	NA	tRNA-Gln	-	533396
19	533352	+	Cj0572	*ribA*	3,4-dihydroxy-2-butanone-4-phosphate synthase	H	533396
20	559819	-	Cj0601c	NA	sodium-dependent transmembrane transportprotein	P	559961
21	650903	-	Cj0693c	NA	rRNA small subunit methyltransferase H	J	650942
22	651019	+	Cj0694	NA	periplasmic protein	O	650942
23	668817	+	Cj0713	*trmD*	tRNA (guanine-N(1)-)-methyltransferase	J	668729

24	784698	-	Cj0835c	*acnB*	aconitate hydratase B	C	784757
25	784713	+	Cj0836	*ogt*	methylated-DNA--protein-cysteinemethyltransferase	H	784757
26	825931	-	Cj0887c	NA	flagellin	N	825941
27	826048	+	Cjp16	NA	tRNA-Leu	-	825941
28	833903	-	Cj0893c	*rpsA*	30S ribosomal protein S1	J	833960
29	854494	-	Cj0917c	*cstA*	integral membrane protein	T	854585
30	878783	-	Cjt04	NA	tRNA-Lys	-	878807
31	901529	-	Cj0961c	*rpmH*	50S ribosomal protein L34	J	901544
32	901608	+	Cj0962	NA	acetyltransferase	K	901544
33	943527	+	Cjp21	NA	tRNA-Arg	-	943586
34	954087	-	Cj1022c	NA	integral membrane protein	S	954014
35	1005092	+	Cj1070	*rpsF*	30S ribosomal protein S6	J	1005131
36	1051049	-	Cj1118c	*cheY*	chemotaxis protein CheY	KT	1051034
37	1109667	-	Cj1182c	*rpsB*	30S ribosomal protein S2	J	1109675
38	1119165	-	Cj1190c	*cetA*	bipartate energy taxis response protein CetA	NT	1119212
39	1154190	-	Cjp22	NA	tRNA-Asn	-	1154324
40	1188925	+	CjNC3	NA	NA	-	1189040
41	1189090	+	Cj1259	*porA*	major outer membrane protein	P	1189040
42	1215039	+	Cjp23	NA	tRNA-Met	-	1214978
43	1252251	+	Cj1324	NA	hypothetical protein	-	1252334
44	1270994	-	Cj1339c	*flaA*	flagellin A	N	1270979
45	1287837	-	Cjp25	NA	tRNA-Ser	-	1287941
46	1290996	-	Cj1358c	*nrfH*	cytochrome c nitrite reductase small subunit	C	1290911
47	1321379	-	Cj1383c	NA	hypothetical protein	-	1321436
48	1354172	-	Cj1420c	NA	methyltransferase	Q	1354040
49	1466181	-	Cj1534c	NA	bacterioferritin	P	1466042
50	1549635	+	Cjp27	NA	tRNA-Gly	-	1549565
51	1554412	-	Cj1625c	*sdaC*	amino acid transporter	E	1554449
52	1564833	+	Cj1639	NA	NifU protein	O	1564877
53	1579989	-	Cj1656c	NA	hypothetical protein	S	1579958
54	1590720	-	Cjp33	NA	tRNA-Pro	-	1590782
55	1626367	-	Cjp34	NA	tRNA-Met	-	1626356
56	1626481	+	Cjt06	NA	tRNA-Ser	-	1626356
57	1632147	-	Cj1719c	*leuA*	2-isopropylmalate synthase	E	1632131
58	1632224	+	Cj1720	NA	hypothetical protein	-	1632131

CjCemR binds to the region of TSS of genes to the following COG groups: energy production and conversion (e.g., *lctP*, *nrfH*, *acnB*) and translation, ribosomal structure and biogenesis (e.g., *rpml*, *rpsU*, *rpsB*) (C, J COGs, respectively), as well as to the tRNA regions (e.g., tRNA-Gln, tRNA-Gly, tRNA-Lys).

Moreover, the ChIP-seq analysis confirmed our previous ChIP-qPCR and EMSA results (Noszka et al. 2024). The binding site upstream of p*gltA* was identified only with MACS3 (not edgeR) (Fig. 2A), albeit the affinity for this position is weak, which was also observed in the ChIP-qPCR (fold enrichment = ~8; Fig. 5C in (Noszka et al. 2024)). Additionally, ChIP-seq indicated that the Cj1608 strongly binds to the p*lctP*, a region (Fig. 2B) previously identified by Sinha et al. with an EMSA assay (Sinha et al. 2024).

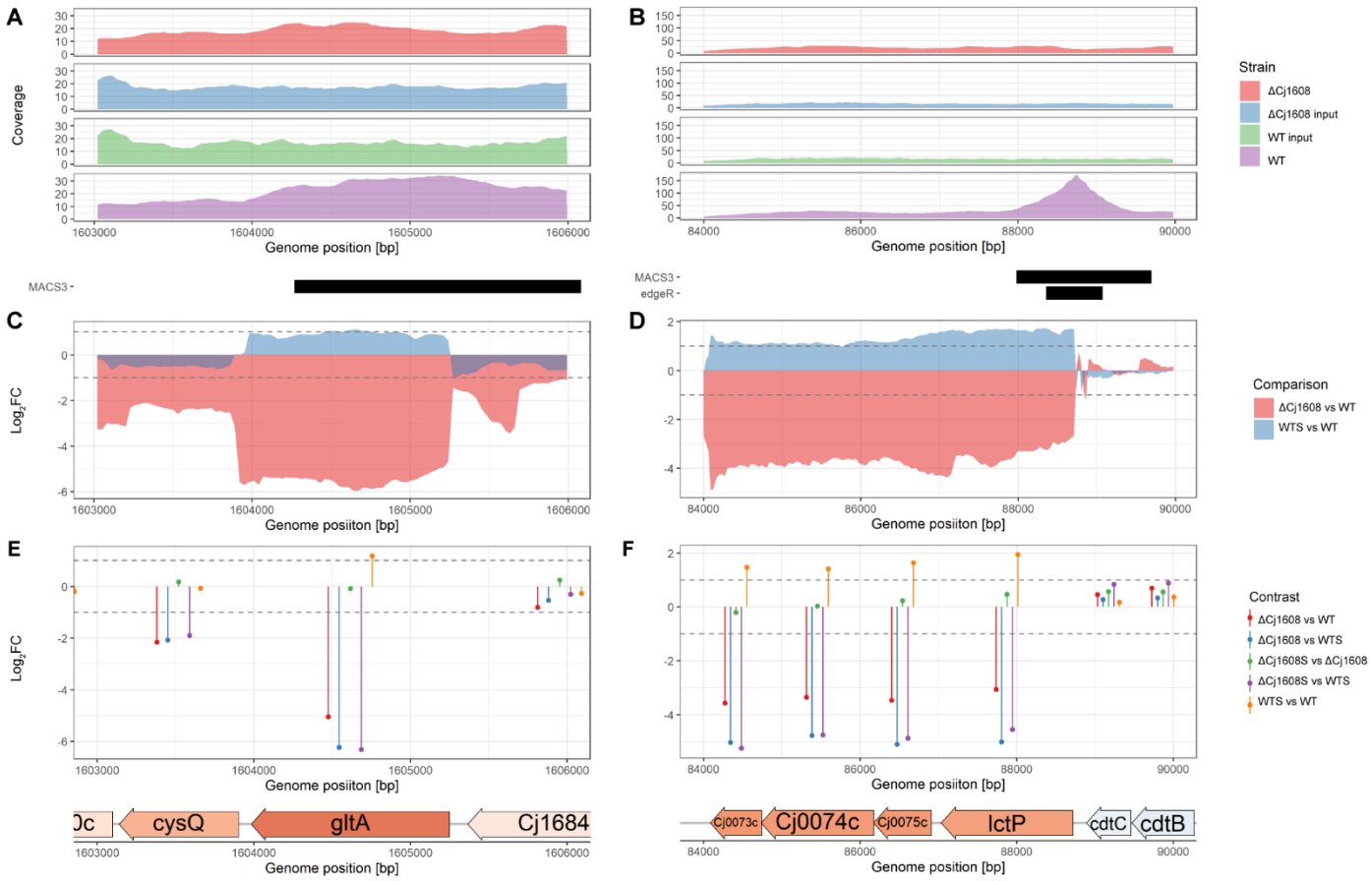

Fig. 2 CjCemR controls *gltA* and *lctP* expression. A and **B** ChIP-seq data profile of the *gltA* and *ltcP*, respectively. Read counts were determined for *C. jejuni* NCTC 11168 wild type (WT) and CjCemR knock-out (ΔCj1608) mutant strains. The y-axis represents the coverage of the DNA reads, while the x-axis represents the position of the genome (in bps). The main peak of the binding site is marked with a thick black line under the x-axis according to the used algorithm. **C** and **D** RNA-seq data profiles of the *gltA* and *lctP*, respectively. The genomic locus *C. jejuni* NCTC 11168 WT and ΔCj1608 mutant strains expression comparison. **E** and **F** contrast comparison between WT, ΔCj1608, WT in oxidative stress conditions (WTS) and ΔCj1608 in oxidative stress conditions (ΔCj1608S) of the RNA-seq data profiles of the *gltA*

and *lctP*, respectively; values above the black dashed lines indicate a change in the expression of $|\log_2 FC| \geq 1$; FDR ≤ 0.05.

In summary, the CjCemR ChIP-seq analysis confirmed previously obtained ChIP-qPCR data and, together with RNA-seq and LC-MS/MS results, indicated that Cj1608 directly activates selected energy production and conversion genes/operons (e.g. *Cj0037c, lctP, icd, acnB*) (Tab. 3). Thus, CjCemR plays a crucial role in metabolism and energy production regulation. The binding of CjCemR to the ChIP-seq-identified binding sites requires further confirmation and functional analyses.

Tab. 3 List of energy production and conversion genes/operons directly activated by CjCemR identified with edgeR and MACS3. FC, fold change; NA, insignificant change (FDR > 0.05); ND, not detected in LC-MS/MS analysis. The RNA-seq and LC-MS/MS of ΔCj1608 vs WT data, according to (Noszka et al. 2024).

locus tag	gene	gene product	FC RNA-seq	FC LC-MS/MS	ChIP best position (bp)
Cj0037c	NA	cytochrome C	NA	0.066	56348
Cj0076c	*lctP*	L-lactate permease	0.119	ND	88820
Cj0531	*icd*	isocitrate dehydrogenase	0.163	0.274	494060
Cj0835c	*acnB*	aconitate hydratase B	0.333	0.171	784757

3.2.2. Identification of AbuCemR (Abu0127) binding sites on *A. butzleri* genome

To identify the AbuCemR binding sites on the *A. butzleri* genome. a ChIP-seq analysis was conducted. *A. butzleri* wild-type (WT) and AbuCemR knock-out mutant (ΔAbu0127) strains were cultivated in a liquid medium at the logarithmic growth phase (OD_{600} = 0.5) under microaerobic conditions, crosslinked, sonicated, and AbuCemR DNA-protein complexes were immunoprecipitated with the polyclonal anti-CemR antibody. Parallel control samples were prepared without immunoprecipitation (input, IN). The ChIP DNA was sequenced with a minimum of 3 million reads with mapping above 85% for each sample. Three biological experiments were performed. Two algorithms, MACS3 (better for broad peaks) and edgeR, were used to identify the binding sites (Fig. 3).

We identified 410 binding sites with MACS3 (17 with fold enrichment >1.5) and 8 with edgeR. Both algorithms identified 6 common binding sites (Tab. 4). The low number of identified peaks resulted from the highly unspecific binding of the anti-AbuCemR antibody (Fig. S12D in (Noszka et al. 2024)).

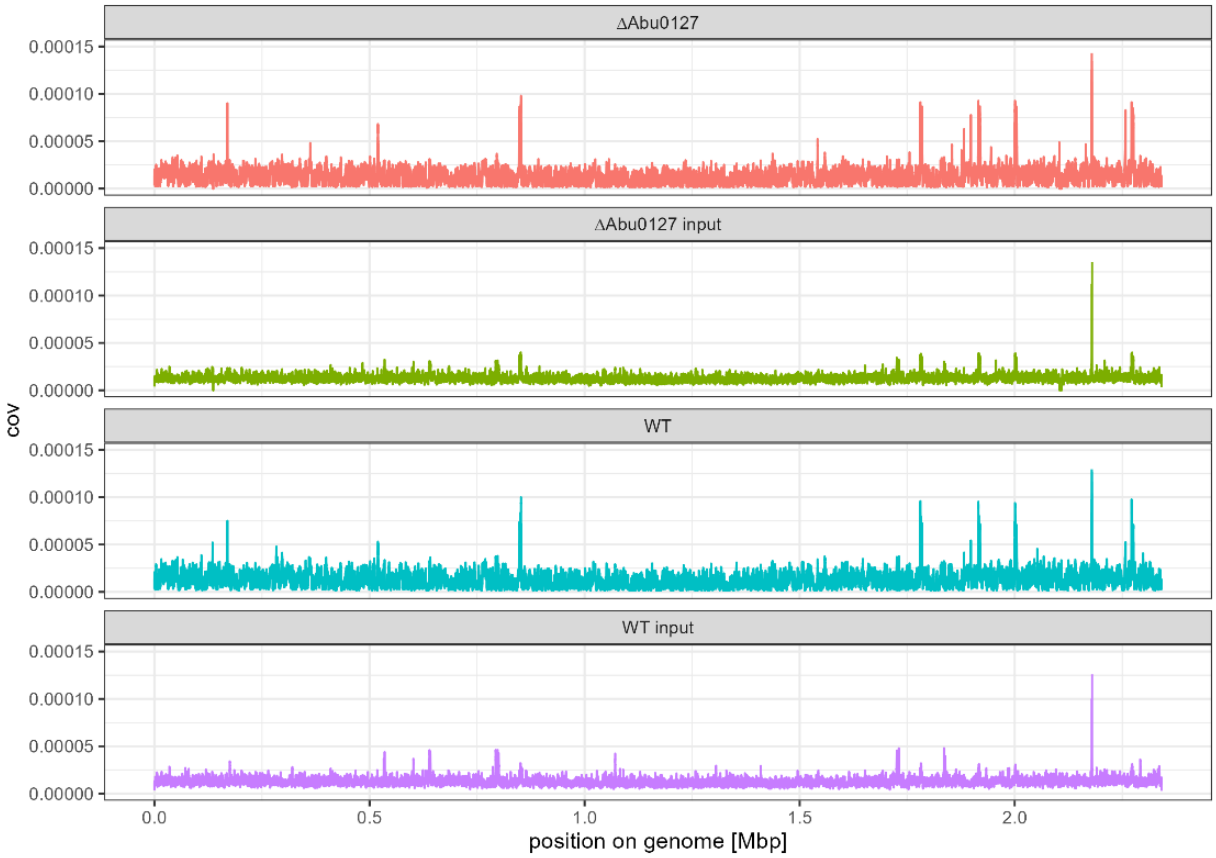

Fig. 3 Identification of AbuCemR binding sites on *A. butzleri* RM4018 genome by ChIP-seq. The ChIP-seq reads of AbuCemR immunoprecipitated samples of *A. butzleri* RM4018 wild type (WT) and AbuCemR knock-out (ΔAbu0127) mutant strains compared with the input DNA samples. COV, read coverage.

Tab. 4 Identification of AbuCemR binding sites on the *A. butzleri* RM4018 genome by ChIP-seq with two algorithms, MACS3 and edgeR. The chromosome position is defined according to the *A. butzleri* RM4018 genome strain (NC_009850.1). FDR, false discovery rate; logFC, log fold change.

ID	ChIP binding start position (bp)	ChIP binding end position (bp)	lenght of the binding site (bp)	ChIP best position (bp)	P-value	FDR	logFC
1	134971	136093	1123	135086	7.02E-13	2.22E-10	2.22
2	137248	137908	661	137330	1.14E-05	1.61E-03	0.77
3	1316239	1316635	397	1316585	6.09E-05	6.43E-03	0.63
4	1610632	1611490	859	1611440	5.86E-07	1.24E-04	0.92
5	2108239	2108767	529	2108354	2.05E-39	2.60E-36	13.75
6	2109097	2109460	364	2109179	4.93E-39	3.13E-36	11.85

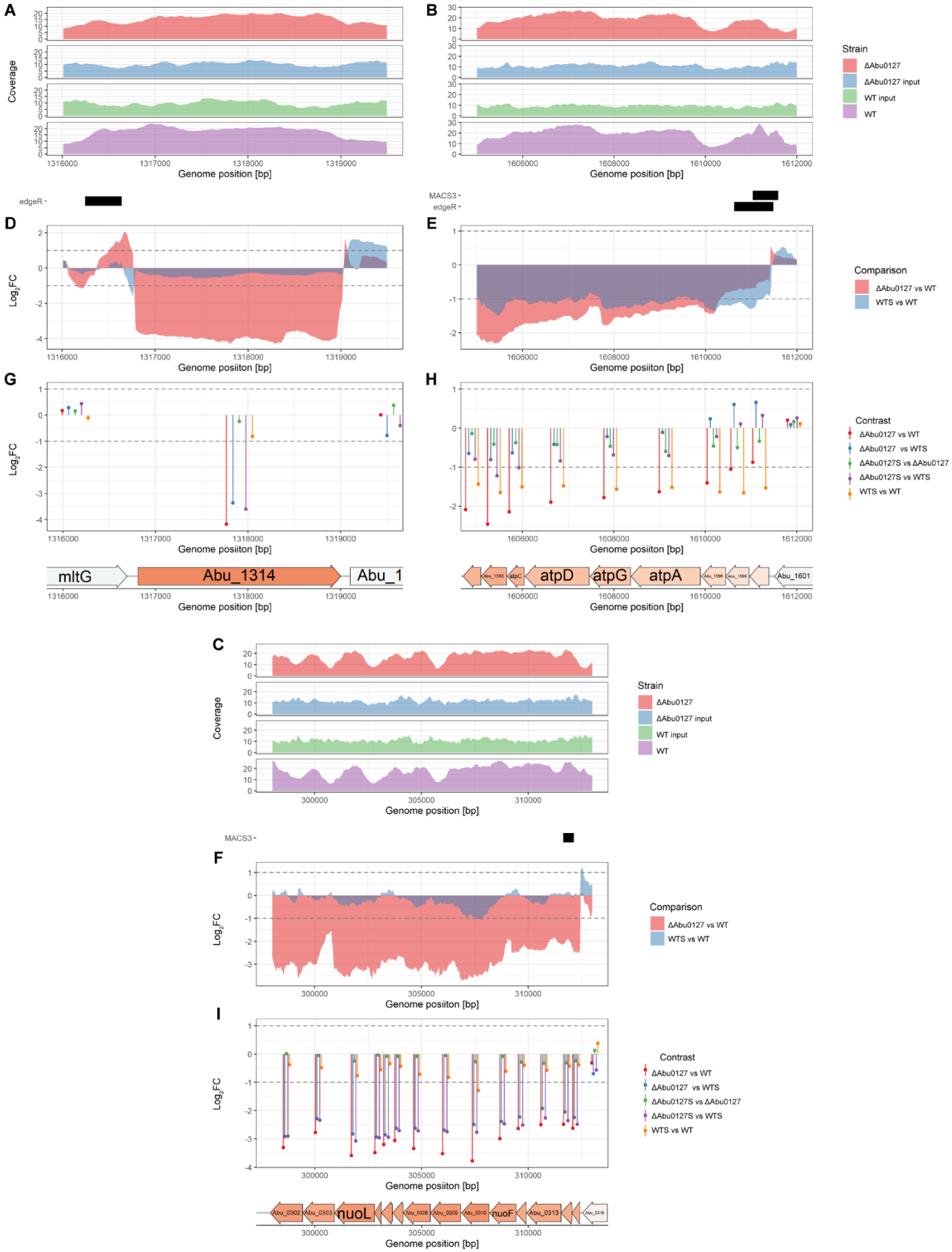

Fig. 4 AbuCemR controls *Abu1314* (*icd*), *atpA* and *nuoA-N* expression.

A, **B** and **C** ChIP-seq data profile of the *Abu1314* (*icd*), *atpA* and *nuoA-N*, respectively. Read counts were determined for *A. butzleri* RM4018 wild type (WT) and AbuCemR knock-out (ΔAbu0127) mutant strains. The y-axis represents the coverage of the DNA reads, while the x-axis represents the position of the genome (in bps). The main peak of the binding site is marked with a thick black line under the x-axis according to the used algorithm. **D**, **E** and **F** RNA-seq data profile of the *Abu1314* (*icd*), *atpA* and *nuoA-N*, respectively. The genomic locus *A. butzleri* RM4018 WT and ΔAbu0127 mutant strains expression comparison. **G**, **H** and **I** contrast the comparison between WT, ΔAbu0127, WT in oxidative stress conditions (WTS) and ΔAbu0127 in oxidative stress conditions (ΔAbu0127S) of the RNA-seq data profiles of the *Abu1314* (*icd*), *atpA* and *nuoA-N*, respectively; values above the black dashed lines indicate a change in the expression of $|\log_2 FC| \geq 1$; FDR ≤ 0.05.

According to the literature, TSSs of *A. butzleri* strains were not defined. However, the AbuCemR binding sites are located in the *icd* (isocitrate dehydrogenase NADP, *Abu1314*) (Tab. 4, ID 3; Fig. 4A) and ATP synthetase genes (*Abu1594-Abu1600*) (Tab. 4, ID 4; Fig. 4B) promotor regions. Both regions are downregulated in the ΔAbu0127 mutant strain, suggesting that Abu0127 directly activates these operons in microaerophilic conditions. Moreover, the MACS3 algorithm (fold enrichment = 1.3) showed a binding site upstream of the previously investigated *nuoA-N* operon (Fig. 4C, (Noszka et al. 2024)). Indeed, this confirms an energy and conservation regulatory function in *A. butzleri* cells. However, due to the poor quality of the antibody, the ChIP-seq assay should be repeated in the future to obtain more reliable data.

4. Conclusions

The main conclusions drawn from the research conducted in this thesis are as follows:

1. CemR proteins are global regulators in the investigated *Campylobacteria* species: *H. pylori*, *C. jejuni*, and *A. butzleri*. As pleiotropic regulators, CemR proteins influence the transcription of more than 30% of genes, affecting many COG groups;

2. CemR is the first discovered regulator redirecting energy conversion pathways in *Campylobacteria*, mainly affecting the expression of the citrate cycle and electron transport chain genes/proteins;

3. CemR controls the metabolic shift related to oxygen availability in *H. pylori*, *C. jejuni* and *A. butzleri* by directly regulating the expression of *gluP*, *gltA* and *nuo* operons, respectively;

4. CemR proteins regulate gene expression in the oxidative stress response processes; CemR is involved in the regulation of catalase (*katA*), alkyl hydroperoxide reductase (*ahpC*) and superoxide dismutase (*sodB*) expression in all investigated species. In *C. jejuni* and *A. butzleri* CemR regulates also peroxide stress regulator (*perR*);

5. CemR controls genes encoding proteins that participate in atypical stress response pathways, e.g., controlling the ComB system responsible for DNA uptake in *H. pylori*.

5. References

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Scientific achievements

Education

I. Doctoral studies at the Wroclaw Doctoral School of Institutes of Polish Academy of Sciences – molecular biology
Supervisor: Anna Pawlik, PhD
Oct 2019 – Sep 2024
Characterisation of CemR regulons of selected pathogenic *Campylobacteria* species.

II. Master studies at the University of Wroclaw – microbiology
Supervisor: Jacek Rybka, PhD
Oct 2017 – Jun 2019
The use of bacterial chromosomal mutants to study the surface structures of *Salmonella*.

III. Bachelor studies at the University of Wroclaw – microbiology
Supervisor: Jacek Rybka, PhD
Oct 2014 – Jul 2017
Methods of bacterial chromosomal mutants construction.

Experience

I. Researcher at the Laboratory of Molecular Biology of Microorganisms, Institute of Immunology and Experimental Therapy of the Polish Academy of Sciences, Wroclaw
June 2024 – present

II. Research Scholar in the OPUS 17 research project funded by the National Science Centre Poland: "The role of HP1021-like orphan response regulators in physiology and virulence of selected pathogenic species of *Epsilonproteobacteria*" – Hirszfeld Institute of Immunology and Experimental Therapy, Wroclaw
Supervisor: Anna Pawlik, PhD
June 2020 – May 2024

III. Research Scholar in the Sonata BIS 3 research project funded by the National Science Centre Poland: "Building bacterial orisome on bipartite origins - structural, functional and regulatory aspects of the process based on the analysis of initiation of *Helicobacter pylori* chromosome replication" – Hirszfeld Institute of Immunology and Experimental Therapy, PAS, Wroclaw

Supervisor: Anna Pawlik, PhD
Oct 2019 – May 2020

IV. Intern in a project "Genotyping of haemolytic and non-haemolytic *Escherichia coli* isolates from various organisms" – Wroclaw University of Environmental and Life Sciences, Wroclaw
Supervisor: Rafał Kolenda, PhD
Jul 2019 – Sep 2019

International internships and courses

I. Internship at the Astrobiology Center (Centro de Astrobiología) National Institute for Aerospace Technology (Instituto Nacional Técnica Aeroespacial), Department of Molecular Evolution, Madrid, Spain; FNP START travel scholarship
Supervisor: José Eduardo González-Pastor, PhD
Oct 2024 (planned)

II. EMBO Practical Course "Synthetic biology in action: beyond standard metabolism" (https://www.embl.org/about/info/course-and-conference-office/events/syn22-01/)
European Molecular Biology Laboratory, Heidelberg (Germany)
11-18 Sep 2022

III. EMBO Virtual course "Introduction to RNA-seq and functional interpretation"
European Molecular Biology Laboratory - European Bioinformatics Institute (UK, online) (https://www.ebi.ac.uk/training/events/introduction-rna-seq-and-functional-interpretation-2022/#vf-tabs__section--tab1)
21-25 Feb 2022

IV. Internship at the Philipp University of Marburg in the Chromosome Biology Group, LOEWE-Center for Synthetic Microbiology, Marburg, Germany; ERASMUS+ travel scholarship
"Synthetic chromosome (SynVic II) incorporation to *Escherichia coli* prime chromosome"
Supervisor: Prof. Dr. Torsten Waldminghaus
July 2018 – Sep 2018

Project leader

I. Principal investigator in the Preludium 21 research project **"**The role of non-coding RNAs in Helicobacter pylori response to oxidative stress and the activity of the HP1021 regulon" founded by the National Science Center Poland, project number 2022/45/N/NZ2/02502, in cooperation with Prof. Dr. Cynthia Sharma from the University of Würzburg (Germany)
Feb 2023 – present

II. Principal investigator in the proteomic sub-project "The characteristic of the regulons of HP1021-like regulators of three pathogenic *Campylobacterales* species: *Helicobacter pylori*, *Campylobacter jejuni* and *Aliarcobacter butzleri*" financed by European Proteomics Infrastructure Consortium EPIC-XS (0000446), project number 823839, funded by the Horizon 2020 programme of the European Union under supervision of Christina Ludwig, PhD from the Bavarian Center for Biomolecular Mass Spectrometry (Germany).
July 2022 – July 2023

Publications

I. **Mateusz Noszka**, Agnieszka Strzałka, Jakub Muraszko, Dirk Hofreuter, Miriam Abele, Christina Ludwig, Kerstin Stingl, Anna Zawilak-Pawlik, *CemR atypical response regulator impacts energy conversion in* Campylobacteria, mSystems, 2024. IF_{2023} 5, pkt MNiSW 140.

II. Eva Krzyżewska-Dudek, Vinaya Dulipati, Katarzyna Kapczyńska, **Mateusz Noszka**, Carmen Chen, Juha Kotimaa, Marta Książczyk, Bartłomiej Dudek, Gabriela Bugla-Płoskońska, Krzysztof Pawlik, Seppo Meri, Jacek Rybka, *Lipopolysaccharide with long O-antigen is crucial for* Salmonella *Enteritidis to evade complement activity and to facilitate bacterial survival in vivo in the* Galleria mellonella *infection model*, Medical Microbiology and Immunology, 213, 8 2024. IF_{2023} 5.5, pkt MNiSW 100.

III. **Mateusz Noszka**, Agnieszka Strzałka, Jakub Muraszko, Rafał Kolenda, Chen Meng, Christina Ludwig, Kerstin Stingl, Anna Zawilak-Pawlik, *Profiling of the* Helicobacter pylori *redox switch HP1021 regulon using a multi-omics approach*, Nature Communications, 14, 6715, 2023. IF_{2023} 14.7, pkt MNiSW 200.

IV. Piotr Szczepanowski*, **Mateusz Noszka***, Dorota Żyła-Uklejewicz, Fabian Pikuła, Malgorzata Nowaczyk-Cieszewska, Artur Krężel, Kerstin Stingl, Anna Zawilak-Pawlik, *HP1021 is a redox switch protein identified in* Helicobacter pylori, Nucleic Acids Research, 49, 12, 2021. IF_{2021} 19.16, pkt MNiSW 200.

V. Adrianna Aleksandrowicz, Muhammad Moman Khan, Katarzyna Sidorczuk, **Mateusz Noszka**, Rafał Kolenda, *Whatever makes them stick–Adhesins of avian pathogenic* Escherichia coli, Veterinary Microbiology, 257, 2021. IF_{2021} 5.6, pkt MNiSW 100.

VI. Rafał Kolenda, Katarzyna Sidorczuk, **Mateusz Noszka**, Adrianna Aleksandrowicz, Muhammad Moman Khan, Michał Burdukiewicz, Derek Pickard, Peter Schierack, *Genome placement of alpha-haemolysin cluster is associated with alpha-haemolysin sequence variation, adhesin and iron acquisition factor profile of* Escherichia coli, Microbial Genomics, 7, 12, 2021. IF_{2021} 5.7, pkt MNiSW 40.

VII. Malgorzata Nowaczyk-Cieszewska, Dorota Zyla-Uklejewicz, **Mateusz Noszka**, Pawel Jaworski, Thorsten Mielke, Anna Magdalena Zawilak-Pawlik, *The role of* Helicobacter pylori *DnaA domain I in orisome assembly on a bipartite origin of chromosome replication*, Molecular Microbiology, 113, 2, 2020. IF_{2020} 3.5, pkt MNiSW 100.

* - equal contribution

Total IF 59.16
Total pkt MNiSW 880

Selected scientific conferences

I. **Mateusz Noszka**, Agnieszka Strzałka, Jakub Muraszko, Dirk Hofreuter, Miriam Abele, Christina Ludwig, Kerstin Stingl, Anna Zawilak-Pawlik, *CemR atypical response regulator impacts energy conversion in* Campylobacteria, 7th Joint Microbiology & Infection Conference of the DGHM and VAMM, Würzburg, Germany; talk
02–05 June 2024

II. **Mateusz Noszka**, Agnieszka Strzałka, Jakub Muraszko, Rafał Kolenda, Chen Meng, Christina Ludwig, Kerstin Stingl, Anna Zawilak-Pawlik, *The tremendous impact of HP1021 on* Helicobacter pylori *cell physiology and response to oxidative stress*, EMBL

Symposium: New approaches and concepts in microbiology, Heidelberg, Germany; poster
27–30 June 2023

III. **Mateusz Noszka**, Agnieszka Strzałka, Jakub Muraszko, Kerstin Stingl, Anna Zawilak-Pawlik; *The regulon of the* Helicobacter pylori *HP1021 redox switch regulator*; 14th International Workshop On Pathogenesis and Host Response in *Helicobacter* Infections, Helsingør, Denmark; talk
29 June 2022 – 02 July 2022

IV. **Mateusz Noszka**, *Target genome editing in bacteria – from one to multiple simultaneous modifications,* Life & Space PTAstrobio, Poland; poster
29 Sep 2021 – 1 Oct 2021

V. **Mateusz Noszka**, Piotr Szczepanowski, Dorota Żyła-Uklejewicz, Fabian Pikuła, Małgorzata Nowaczyk-Cieszewska, Artur Krężel, Kerstin Stingl, Anna Zawilak-Pawlik; *HP1021 is the first redox switch protein identified in* Helicobacter pylori; World Microbe Forum 2021 – ASM and FEMS collaboration; talk
20–24 June 2021

VI. **Mateusz Noszka**, Piotr Szczepanowski, Dorota Żyła-Uklejewicz, Fabian Pikuła, Małgorzata Nowaczyk-Cieszewska, Artur Krężel, Kerstin Stingl, Anna Zawilak-Pawlik; *HP1021 is the first redox switch protein identified in* Helicobacter pylori; MICOM 2021, Jena, Germany; talk
29–31 Mar 2021

Most important honours & awards

I. FNP START 2024 scholarship of the Foundation for Polish Science for the outstanding young scientists in Poland

II. Ludwik Hirszfeld scholarship in the field of biological and medical sciences (President of Wroclaw scholarship) for the 2022/2023 academic year

III. Scholarship of the Minister of Science and Education for significant achievements for students for the 2018/2019 academic year

Appendix

This CD includes the Supplementary Data from the papers:

I. Mateusz Noszka, Agnieszka Strzałka, Jakub Muraszko, Rafał Kolenda, Chen Meng, Christina Ludwig, Kerstin Stingl, Anna Zawilak-Pawlik, Profiling of the *Helicobacter pylori* redox switch HP1021 regulon using a multi-omics approach. Nature Communications, 14, 6715, 2023.

II. Mateusz Noszka, Agnieszka Strzałka, Jakub Muraszko, Dirk Hofreuter, Miriam Abele, Christina Ludwig, Kerstin Stingl, Anna Zawilak-Pawlik, CemR atypical response regulator impacts energy conversion in *Campylobacteria*. mSystems, 9, 8, 2024.