

# ASSOCIATION OF *CAMPYLOBACTER JEJUNI* SSP. *JEJUNI* CHEMOTAXIS RECEPTOR GENES WITH MULTILOCUS SEQUENCE TYPES AND SOURCE OF ISOLATION

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*Campylobacter jejuni*'s flagellar locomotion is controlled by eleven chemoreceptors. Assessment of the distribution of the relevant chemoreceptor genes in the *C. jejuni* genomes deposited in the National Center for Biotechnology Information (NCBI) database led to the identification of two previously unknown *tlp* genes and a *tlp5* pseudogene. These two chemoreceptor genes share the same locus in the *C. jejuni* genome with *tlp4* and *tlp11*, but the gene region encoding the periplasmic ligand binding domain differs significantly from other chemoreceptor genes. Hence, they were named *tlp12* and *tlp13*.

Consequently, it was of interest to study their distribution in *C. jejuni* subpopulations of different clonality, and their cooccurrence with the eleven previously reported chemoreceptor genes. Therefore, the presence of all *tlp* genes was detected by polymerase chain reaction (PCR) in 292 multilocus sequence typing (MLST)-typed *C. jejuni* isolates from different hosts.

The findings show interesting trends: *Tlp4*, *tlp11*, *tlp12*, and *tlp13* appeared to be mutually exclusive and cooccur in a minor subset of isolates. *Tlp4* was found to be present in only 33.56% of all tested isolates and was significantly less often detected in turkey isolates. *Tlp11* was tested positive in only 17.8% of the isolates, while *tlp12* was detected in 29.5% of all isolates, and *tlp13* was found to be present in 38.7%.

**Keywords:** *Campylobacter jejuni*, MLST, chemotaxis receptors, transducer-like proteins, Tlp4, Tlp5, Tlp7, Tlp11, Tlp12, Tlp13

**Abbreviations:** CC, clonal complex; CcaA, *Campylobacter* chemoreceptor for aspartate A (Tlp1); CcmL, *Campylobacter* chemoreceptor for multiple ligands (Tlp3); *cj*, gene numbering based on the genome sequence of *C. jejuni* strain NCTC 11168; MCP, methyl-accepting chemotaxis protein; MLST, multilocus sequence typing; ST, sequence type; *tlp*, transducer-like protein gene; UPGMA, unweighted-pair group method using average linkages

## Introduction

*Campylobacter jejuni* ssp. *jejuni* is the most prevalent bacterial pathogen that causes acute enteritis in industrialized nations among human beings. This infection is known as campylobacteriosis and is characterized by watery or bloody diarrhea, fever, and abdominal cramps [1, 2]. In consequence of campylobacteriosis, postinfectious sequelae, namely, the Guillain–Barré syndrome, inflammatory bowel disease, and reactive arthritis may occur [3, 4].

*C. jejuni* ssp. *jejuni* has a wide range of hosts. These include wild birds and farm animals, namely, poultry, cattle, sheep, and, rarely, swine. It can also be found as contaminant in milk, meat, and surface water, but most studies have concluded that poultry is the natural host of *C. jejuni* ssp. *jejuni* and, hence, the major source of transmission to human beings and other farm animals [5].

Presently, it has been established that, upon entering the intestine of a host, *C. jejuni* ssp. *jejuni* has to overcome the intestinal microbiota barrier covering the epithelial cells and the mucin layer for successful colonization [6]. This

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is driven by an inherent necessity to find niches with suitable optimal growth conditions such as amino acids and carboxylic acids which are the major carbon sources for *C. jejuni* ssp. *jejuni* [7]. In order to locate and reach these niches, *C. jejuni* ssp. *jejuni* utilizes a complex chemotaxis system, which controls its locomotion mediated by usually two flagella. This flagellar locomotion is controlled by different chemoreceptors comprising of – up to date – 13 transducer-like proteins (Tlp) and two aerotaxis genes [8–10]. These chemoreceptors are categorized into three groups, namely, A, B, and C [8].

Group A receptors consist of a periplasmic ligand binding domain and a cytoplasmic signaling domain. Members of this group include: Tlp1, Tlp2, Tlp3, Tlp4, Tlp7, Tlp10, and Tlp11. Group B consists of one receptor, Tlp9, with two cytoplasmic ligand–proteins sensing aerotactic signals (Aer1 and Aer2). Group C receptors miss a periplasmic ligand-binding domain but contain a cytoplasmic signaling domain homologous to that of group A receptors. Their role is to sense internal cytoplasmic signals. Members of this group include Tlp5, Tlp6, Tlp8, and Tlp7<sub>c</sub> [9, 11]. To date, the chemoeffectors and the interaction mechanisms of the various receptors are only partially understood. The identification of specific chemoeffectors is complicated by the phenomenon where one chemoreceptor compensates the function of the other [12].

In spite of this challenge, a few chemoeffectors have been successfully identified: L-aspartate acts as a chemoattractant sensed by Tlp1 and, hence, is named *Campylobacter* chemoreceptor for aspartate A (CcaA) [13], and formic acid has been identified as chemoattractant of both variants of Tlp7 [11]. In the majority of *C. jejuni* ssp. *jejuni* isolates, for example in strain 81-176, the *tlp7* gene is translated as one RNA and expressed as one 58 kDa protein. In contrast to that in particular *C. jejuni* ssp. *jejuni* isolates, for example in strain NCTC 11168, the *tlp7* chemoreceptor gene is interrupted by a stop codon (at position 891 663 of the NCTC 11168 genome). This stop codon splits the gene in two open reading frames (ORFs), *cj0952c* and *cj0951c*, which are both transcribed and expressed as single proteins (25 kDa + 33 kDa). Only if both proteins Cj0952c and Cj0951c are expressed and interact as heterodimer, a chemotactic signal towards formic acid is sensed [11, 14]. To distinguish the three proteins, the cytoplasmic protein Cj0951c was named Tlp7<sub>c</sub>, protein Cj0952c containing the membrane-spanning regions, Tlp7<sub>m</sub>; and the 58 kDa protein containing the cytoplasmic as well as the membrane-spanning regions, Tlp7<sub>mc</sub> [9]. Bile acids generally act as chemorepellents and are sensed by Tlp3 and Tlp4 [15]. It has been demonstrated that Tlp3 binds the chemoattractants isoleucine, purine, malic acid, and fumaric acid and the chemorepellents lysine, glucosamine, succinic acid, arginine, and thiamine. Therefore, it has been named *Campylobacter* chemoreceptor for multiple ligands (CcmL) [16]. Tlp9/CetA (Cet is an abbreviation for *Campylobacter* energy taxis), lacking an own specific ligand-binding domain, acts as redox-sensing receptor together with its two cytoplasmic ligand–proteins Aer1/CetC and Aer2/CetB,

which are homologues of cytoplasmic redox-sensing proteins (Per-ARNT-Sim [PAS] domains) [8]. CetABC acts as energy taxis system driving *C. jejuni* cells towards high redox potentials and favorable conditions for energy generation, respectively. In contrast to CetABC, the group C receptor Tlp8 (CetZ), a cytoplasmic sensor with two PAS domains, acts as an opponent to CetABC driving cells away from high redox potentials [17].

In this study, we analyzed the currently known chemoreceptor genes in 142 *C. jejuni* ssp. *jejuni* genome sequences which have been deposited in the NCBI genome database (32 complete genomes and 110 draft genomes in February 2015) and discovered two new unreported chemoreceptor genes, which we have named *tlp12* and *tlp13* as well as a *tlp5* pseudogene.

In addition, we evaluated the distribution of *tlp12*, *tlp13*, the *tlp5* pseudogene, and the 15 currently known chemoreceptor genes in 292 *C. jejuni* ssp. *jejuni* strains that were isolated from humans, chicken, bovine, and turkey.

The phylogenetic relationship of these isolates was ascertained by multilocus sequence typing (MLST) followed by statistical analysis of the occurrence of the *C. jejuni* ssp. *jejuni* chemoreceptors in each clonal complex.

## Materials and methods

### *Bacterial isolates and culture conditions*

In this study, a total of 292 *C. jejuni* ssp. *jejuni* isolates (150 of human, 68 of chicken, 43 of bovine, 24 of turkey, three of ovine, two of wild bird, two of canine, and one of riparian origin) were included. The human isolates were isolated from stool samples of suspected cases of campylobacteriosis reported at the University Medical Center Göttingen, Germany during the period of 2000–2004. Ethical clearance for the analysis was obtained from the Ethics Committee at the University Medical Center Göttingen, Germany. However, these bacterial isolates have been used in some preliminary studies [14, 18–20]. Hence, no evaluation including personal patient data was performed; the Ethics Committee at the University Medical Center Göttingen waived the need for written informed consent from the donor or the next of kin. The study design, therefore, corresponds to a retrospective data analysis.

The chicken, bovine, and turkey isolates were obtained from the Bundesinstitut für Risikobewertung (BfR, Federal Institute for Risk Assessment, [http://www.bfr.bund.de/de/nationales\\_referenzlabor\\_fuer\\_campylobacter-8818.html](http://www.bfr.bund.de/de/nationales_referenzlabor_fuer_campylobacter-8818.html)) in Berlin, Germany.

Species identification was performed using the MALDI Biotyper system (Bruker Daltonics, Bremen, Germany). Results with MALDI Biotyper identification score values  $\geq 2.000$  were considered correct. Additionally, multiplex polymerase chain reaction (PCR) was used to discriminate between *C. jejuni* and *Campylobacter coli* [21]. No isolates, featuring *C. coli* or *C. jejuni* ssp. *doylei* characteristic

MLST clonal complexes/sequence types, were used this study.

The *C. jejuni* ssp. *jejuni* isolates were cultured on Columbia agar base (Merck) supplemented with 5% sheep blood (BA) and incubated at 42 °C under microaerophilic conditions (5% O<sub>2</sub>, 10% CO<sub>2</sub>, 85% N<sub>2</sub>) for 18 h prior to DNA extraction.

#### DNA extraction

Genomic DNA of all *C. jejuni* ssp. *jejuni* isolates was extracted using the QIAamp DNA Mini Kit (Qiagen) ac-

cording to the manufacturer's instructions. Genomic DNA concentration was measured using a nanodrop 1000 spectrophotometer (peqlab, Thermo Fisher Scientific, Wilmington, USA).

#### Evaluation of chemoreceptor distribution in relation to source of isolation and clonal complex PCR conditions

For analysis of *tlp* and aerotaxis genes in each isolate based on source of isolation and clonal complex, 50 ng DNA and 1 µL of 10 µM of primers listed in Table 1 were used. Primers to amplify the genes of *tlp1–3* and *tlp5–10*

**Table 1.** Primers used for amplification of *tlp* genes

Gene	Primer name	Sequence 5'–3'	Annealing temp. (°C)	Length (bp)
<i>tlp1/ccaA</i> <i>cj1506</i>	tlp1-F02	AGCTAATCTGCAAGTTGTGCAAG	60.0	1382
	tlp1-R02	CCGCAAGCTGTCTTACCTCA		
<i>tlp2</i> <i>cj0144</i>	tlp2-F03	AAGAATTTTAGAGATGCTGGAAGA	59.0	1191
	tlp2-R03	AGTGGTTAAGCTTTGAACAGCA		
<i>tlp3/ccmL</i> <i>cj1564</i>	tlp3-F06	CGTTGAAGATTTCCGTTCCAC	59.0	649
	tlp3-R06	AGCGCTTTCGGTAATACAAGC		
<i>tlp4/12-consensus</i>	tlp4/12c-F03	TGGGTTGGAATTTAGTTGTATT	58.0	1658
	tlp4/12c-R03	CCTCTACCATGTTCTCCAGC		
<i>tlp4</i> <i>cj0262c</i>	tlp4-F01	TCGCCAATGCAATCAAAGCA	58.0	1015
	tlp4/12c-R03	CCTCTACCATGTTCTCCAGC		
<i>tlp5-consensus</i> <i>cj0246</i>	tlp5c-F02	GGAATTGCAAAAAGTTTAGTGCC	57.0	792/207*
	tlp5c-R02	AGCTAGAACAATCTTCATAACATTTG		
<i>tlp6</i> <i>cj0448</i>	tlp6-F01	GCAGGTGAACATGGGCGTGGT	59.0	567
	tlp6-R01	CGATGCATTTTCAGCAACTTCGCA		
<i>tlp7<sup>†</sup>/ccfA</i> <i>cj0951c/cj0952c</i>	tlp7-F01	AGGTTTCTGCTGCAATTTTGTGGTG	53.0	880
	tlp7-R01	AGCAAGTCTCCAAGTTCATTGCCA		
<i>tlp8</i> <i>cj1110</i>	tlp8-F01	TGCTGCTGCTAATCGTTCTATGGC	58.0	597
	tlp8-R01	GCACGTGCTGCCTCAATAGCA		
<i>tlp9/cetA</i> <i>cj1190</i>	cetA-F01	TCGTAAGGCTTTGCCTGAAGGT	58.0	470
	cetA-R01	CCGCAAAGCCCCTACCATGC		
<i>tlp9/cetB/aer2</i> <i>cj1189</i>	cetB-F01	TGCAGGTTATACCATGGGTGAAGTT	57.0	308
	cetB-R01	AGCCTTGTTGCTGTTCTGCTCTT		
<i>tlp9/aer1</i> <i>cj1191</i>	aer1-F01	ACATGAAGATATGCCACGCACTGT	57.0	186
	aer1-R01	GGTGCACGACGAACAGAATAA		
<i>tlp10</i> <i>cj0019</i>	tlp10-F01	AGAAGCCAATCTACACTCTCGTT	56.0	532
	tlp10-R01	AAATCCACGCCCATGTTTCGC		
<i>tlp11</i>	tlp11a-F02	AGCAATAGGAATAGTCTTAGGCAT	59.0	1770
	tlp11/13c-R02	GCACGAGCTGCTTCAATAGC		
<i>tlp12</i>	tlp12-F01	TCGCCAATGCAATCAAAGCA	58.0	1015
	tlp4/12c-R03	CCTCTACCATGTTCTCCAGC		
<i>tlp13</i>	tlp13-F01	TCGAGCGTTAGTTCAAAACTCT	59.0	1185
	tlp13-short-R01	ACCCATTTGCCCAATTCATCA		

\*Primers amplify both *tlp5* variants. The intact gene results in an amplicon size of 792 bp. The disrupted gene results in an amplicon size of 207 bp

†The interrupting stop codon was detected by *AseI* restriction of the amplicon

**Table 2.** Percentage distributions of *tlp4*, *tlp5*, *tlp7m*, *tlp11*, *tlp12*, and *tlp13* among 292 *C. jejuni* isolates and their associations with host and CC/ST

Host or CC/ST	No. of isolates with chemoreceptor gene/total no. (%)					
	<i>tlp4</i>	<i>tlp5</i>	<i>tlp7<sub>m</sub></i>	<i>tlp11</i>	<i>tlp12</i>	<i>tlp13</i>
<b>Host</b>						
All	98/292 (33.6)	165/292 (56.5)	67/292 (22.9)	52/292 (17.8)	86/292 (29.5)	113/292 (38.7)
Human	61/150 (40.7)	84/150 (56.0)	30/150 (20.0)	20/150 (13.3)	51/150 (34.0)	69/150 (46.0) <sup>#</sup>
Chicken	18/68 (26.5)	35/68 (51.5)	<b>4/68 (5.9)*</b>	4/68 (5.9)*	23/68 (33.8)	32/68 (47.1)
Bovine	14/43 (32.6)	<b>31/43 (72.1)<sup>#</sup></b>	<b>27/43 (62.8)*</b>	<b>24/43 (55.8)*</b>	<b>5/43 (11.6)<sup>#</sup></b>	<b>2/43 (4.7)*</b>
Turkey	<b>2/24 (8.3)*</b>	9/24 (20.1)	5/24 (20.8)	3/24 (12.5)	7/24 (29.2)	9/24 (37.5)
Other hosts	3/7 (42.9)	6/7 (85.7)	1/7 (14.3)	1/7 (14.3)	0/7 (0.0)	1/7 (14.3)
CC21	21/84 (25.0)	<b>84/84 (100)*</b>	50/84 (59.5) <sup>*</sup>	<b>35/84 (41.7)*</b>	32/84 (38.1)	17/84(20.2) <sup>*</sup>
ST21	10/31 (32.3)	<b>31/31 (100)*</b>	<b>30/31 (96.8)*</b>	<b>23/31 (74.2)*</b>	0/31 (0.0) <sup>*</sup>	2/31 (6.5) <sup>*</sup>
ST53	1/8 (12.5)	<b>8/8 (100)*</b>	<b>8/8 (100)*</b>	<b>7/8 (87.5)*</b>	0/8 (0.0) <sup>*</sup>	<b>7/8 (87.5)*</b>
ST50	2/17 (11.8)	<b>17/17 (100)*</b>	1/17 (5.9)	0/17 (0.0) <sup>*</sup>	<b>17/17 (100)*</b>	5/17 (29.4)
Other ST	8/28 (28.6)	<b>28/28 (100)*</b>	11/28 (39.3)	5/28 (17.9)	15/28 (53.6)	3/28 (10.7)
CC52	<b>5/6 (83.3)<sup>#</sup></b>	1/6 (16.7)	1/6 (16.7)	0/6 (0.0) <sup>*</sup>	0/6 (0.0) <sup>*</sup>	<b>5/6 (83.3)<sup>#</sup></b>
CC446	1/5 (20.0)	0/5 (0.0) <sup>*</sup>	0/5 (0.0) <sup>*</sup>	0/5 (0.0) <sup>*</sup>	0/5 (0.0) <sup>*</sup>	<b>5/5 (100)*</b>
CC49	<b>4/5 (80.0)<sup>#</sup></b>	0/5 (0.0) <sup>*</sup>	0/5 (0.0) <sup>*</sup>	0/5 (0.0) <sup>*</sup>	1/5 (20.0)	1/5 (20.0)
CC1034	0/7 (0.0) <sup>*</sup>	0/7 (0.0) <sup>*</sup>	0/7 (0.0) <sup>*</sup>	0/7 (0.0) <sup>*</sup>	3/7 (42.9)	4/7 (57.1)
CC354	0/6 (0.0) <sup>*</sup>	0/6 (0.0) <sup>*</sup>	0/6 (0.0) <sup>*</sup>	0/6 (0.0) <sup>*</sup>	<b>5/6 (83.3)<sup>#</sup></b>	3/6 (50)
CC443	1/5 (20.0)	0/5 (0.0) <sup>*</sup>	0/5 (0.0) <sup>*</sup>	0/5 (0.0) <sup>*</sup>	1/5 (20.0)	3/5 (60.0)
CC206	12/21 (57.1) <sup>#</sup>	14/21 (66.7) <sup>#</sup>	0/21 (0.0) <sup>*</sup>	0/21 (0.0) <sup>*</sup>	9/21 (42.9)	6/21 (28.6)
ST46	0/3 (0.0) <sup>*</sup>	<b>3/3(100)*</b>	0/3 (0.0) <sup>*</sup>	0/3 (0.0) <sup>*</sup>	2/3 (66.7)	1/3 (33.3)
ST122	<b>5/5 (100)*</b>	<b>5/5 (100)*</b>	0/5 (0.0) <sup>*</sup>	0/5 (0.0) <sup>*</sup>	2/5 (40.0)	1/5 (20.0)
ST572	<b>5/5 (100)*</b>	0/5 (0.0) <sup>*</sup>	0/5 (0.0) <sup>*</sup>	0/5 (0.0) <sup>*</sup>	0/5 (0.0) <sup>*</sup>	0/5 (0.0) <sup>*</sup>
ST2192	0/3 (0.0) <sup>*</sup>	<b>3/3(100)*</b>	0/3 (0.0) <sup>*</sup>	0/3 (0.0) <sup>*</sup>	<b>3/3 (100)*</b>	2/3 (66.7)
Other ST	2/5 (40.0)	3/5 (60.0)	0/5 (0.0) <sup>*</sup>	0/5 (0.0) <sup>*</sup>	2/5 (40.0)	2/5 (40.0)
CC48	15/20 (75.0)	4/20 (20.0)	4/20 (20.0)	5/20 (25.0)	0/20 (0.0) <sup>*</sup>	1/20 (5.0) <sup>*</sup>
ST38	0/3 (0.0) <sup>*</sup>	<b>3/3 (100)</b>	<b>3/3 (100)</b>	<b>3/3 (100)</b>	0/3 (0.0) <sup>*</sup>	0/3 (0.0) <sup>*</sup>
ST48	<b>7/7 (100)*</b>	0/7 (0.0) <sup>*</sup>	0/7 (0.0) <sup>*</sup>	0/7 (0.0) <sup>*</sup>	0/7 (0.0) <sup>*</sup>	1/7 (14.3)
Other ST	8/10 (80.0)	1/10 (10.0)	1/10 (10.0)	2/10 (20.0)	0/10 (0.0) <sup>*</sup>	0/10 (0.0) <sup>*</sup>
CC257	0/10 (0.0) <sup>*</sup>	0/10 (0.0) <sup>*</sup>	0/10 (0.0) <sup>*</sup>	0/10 (0.0) <sup>*</sup>	<b>8/10 (80.0)<sup>#</sup></b>	<b>9/10 (90)*</b>
CC464	1/8 (12.5)	0/8 (0.0) <sup>*</sup>	0/8 (0.0) <sup>*</sup>	0/8 (0.0) <sup>*</sup>	<b>5/8 (62.5)</b>	<b>8/8 (100)*</b>

Table 2. (cont. d)

Host or CC/ST	No. of isolates with chemoreceptor gene/total no. (%)						
	<i>tlp4</i>	<i>tlp5</i>	<i>tlp7<sub>m</sub></i>	<i>tlp11</i>	<i>tlp12</i>	<i>tlp13</i>	
CC353	0/8 (0.0)*	0/8 (0.0)*	0/8 (0.0)*	0/8 (0.0)*	1/8 (12.5)	1/8 (12.5)	
CC658	1/4 (25.0)	1/4 (25.0)	0/4 (0.0)*	0/4 (0.0)*	0/4 (0.0)*	<b>4/4 (100)*</b>	
CC22	<b>5/9 (55.6)</b>	<b>9/9 (100)*</b>	0/9 (0.0)*	2/9 (22.2)	2/9 (22.2)	<b>5/9 (55.6)</b>	
CC1332	0/2 (0.0)*	0/2 (0.0)*	0/2 (0.0)*	0/2 (0.0)*	<b>2/2 (100)*</b>	<b>2/2 (100)*</b>	
CC45	<b>19/33 (57.6)*</b>	<b>33/33 (100)*</b>	0/33 (0.0)*	0/33 (0.0)*	4/33 (12.1)	12/33 (36.4)	
CC283	<b>5/6 (83.3)#</b>	<b>6/6 (100)*</b>	0/6 (0.0)*	0/6 (0.0)*	0/6 (0.0)*	2/6 (33.3)	
CC42	<b>6/7 (85.7)#</b>	<b>7/7 (100)*</b>	1/7 (14.3)	0/7 (0.0)*	0/7 (0.0)*	1/7 (14.3)	
CC61	0/11 (0.0)*	0/11 (0.0)*	<b>10/11 (90.9)*</b>	<b>10/11 (90.9)*</b>	2/11 (18.2)	0/11 (0.0)*	
Other	2/35 (5.7)	6/35 (17.1)	1/35 (2.3)	0/35 (0.0)	11/35 (31.4)	24/35 (68.6)	

Chemoreceptor genes: *tlp4*, transducer-like protein 4; *tlp5*, transducer-like protein 5 (intact gene); *tlp7<sub>m</sub>*, transducer-like protein 7 membrane associated part (homologue to *cj0952c*); *tlp11*, transducer-like protein 11; *tlp12*, transducer-like protein 12; *tlp13*, transducer-like protein 13; other hosts: include three isolates of ovine, two of wild bird, two of canine, and one of riparian origin; other ST: include ST belonging to the particular CC not listed separately; other: include singletons and CCs with a very low number of isolates included in the study  
<sup>#</sup>*p* < 0.05; \**p* < 0.001 significance level in comparison to the remaining isolates not belonging to the corresponding clonal complexes/sequence types. Additionally, the values in isolate groups with above average numbers of receptor gene positive or negative isolates are given in bold numbers

have been designed to bind to consensus sequences of the genes according to the sequences deposited in the NCBI database. *Tlp2* and *tlp3* primer pairs were designed to bind specifically in the gene regions encoding the periplasmic sensory domain because the cytosolic signaling domains of these chemoreceptors are almost identical; also to *tlp4*, *tlp11*, *tlp12*, and *tlp13*.

Forward primers for *tlp4*, *tlp11*, *tlp12*, and *tlp13* were designed to bind to a region specific for the particular variant, while the consensus reverse primer binds in the highly conserved cytoplasmic signaling region. Additionally, a higher specificity was achieved in the case of *tlp13* using a reverse primer binding in a *tlp13* specific region of the periplasmic region (*tlp13c*-short-R01), confirming the results with the consensus reverse primer.

*Tlp5* specific primers were designed to bind consensus regions of intact *tlp5* as well as of the disrupted gene. In the case of an intact *tlp5* gene, the amplicon size is 792 bp, while in the case of a disrupted gene, the amplicon size is 207 bp.

Differentiation between uninterrupted *tlp7* (*tlp7<sub>mc</sub>*) and the two ORF variant of *tlp7* (*tlp7<sub>m</sub>* and *tlp7<sub>c</sub>*) was performed by *AseI* restriction of the amplicon, as described before [14]. *AseI* cuts the amplicon directly at the stop codon between *tlp7<sub>m</sub>* and *tlp7<sub>c</sub>*. The cycling conditions were 94 °C for 1 min, followed by 35 cycles of 94 °C for 30 s, annealing according to Table 1 for 30 s, and 72 °C for 30 s, with a final elongation of 72 °C for 5 min.

#### MLST and phylogenetic analysis

The MLS-type was established using amplification and sequencing primers reported before [22]. The cycling conditions were 94 °C for 1 min, followed by 35 cycles of 94 °C for 120 s, 50 °C for 60 s, and 72 °C for 60 s, followed by a final elongation step of 72 °C for 5 min [22]. Amplicons of the seven genes included in the *C. jejuni/C. coli* MLST scheme were sent for sequencing to SeqLab Sequence Laboratories GmbH (Göttingen, Germany) using 10 pmol of the respective sequencing primer.

MEGA6 software was used to construct the unweighted pair group method using average linkages (UPGMA)-tree [23]. The *C. jejuni* MLST website (<http://pubmlst.org/campylobacter/>) was consulted for assignment of sequence types and clonal complexes [24].

#### Statistical analyses and multiple sequence alignment

The Statistica software (Statsoft, Tulsa, Oklahoma, USA) was used to perform statistical analysis. The  $\chi^2$  test was used to test for significant differences in the frequencies of the *tlp* genes within the defined groups. The obtained *p* values are shown in Table 2.

Multiple sequence alignment of the *tlp* gene sequences was performed using the Clustal Omega package (<http://www.ebi.ac.uk/Tools/msa/clustalo/>) [25] hosted at the EMBL-EBI bioinformatics web and programmatic tools framework website [26].

## Results

### Analysis of chemoreceptor genes in *C. jejuni* ssp. *jejuni* genome sequences

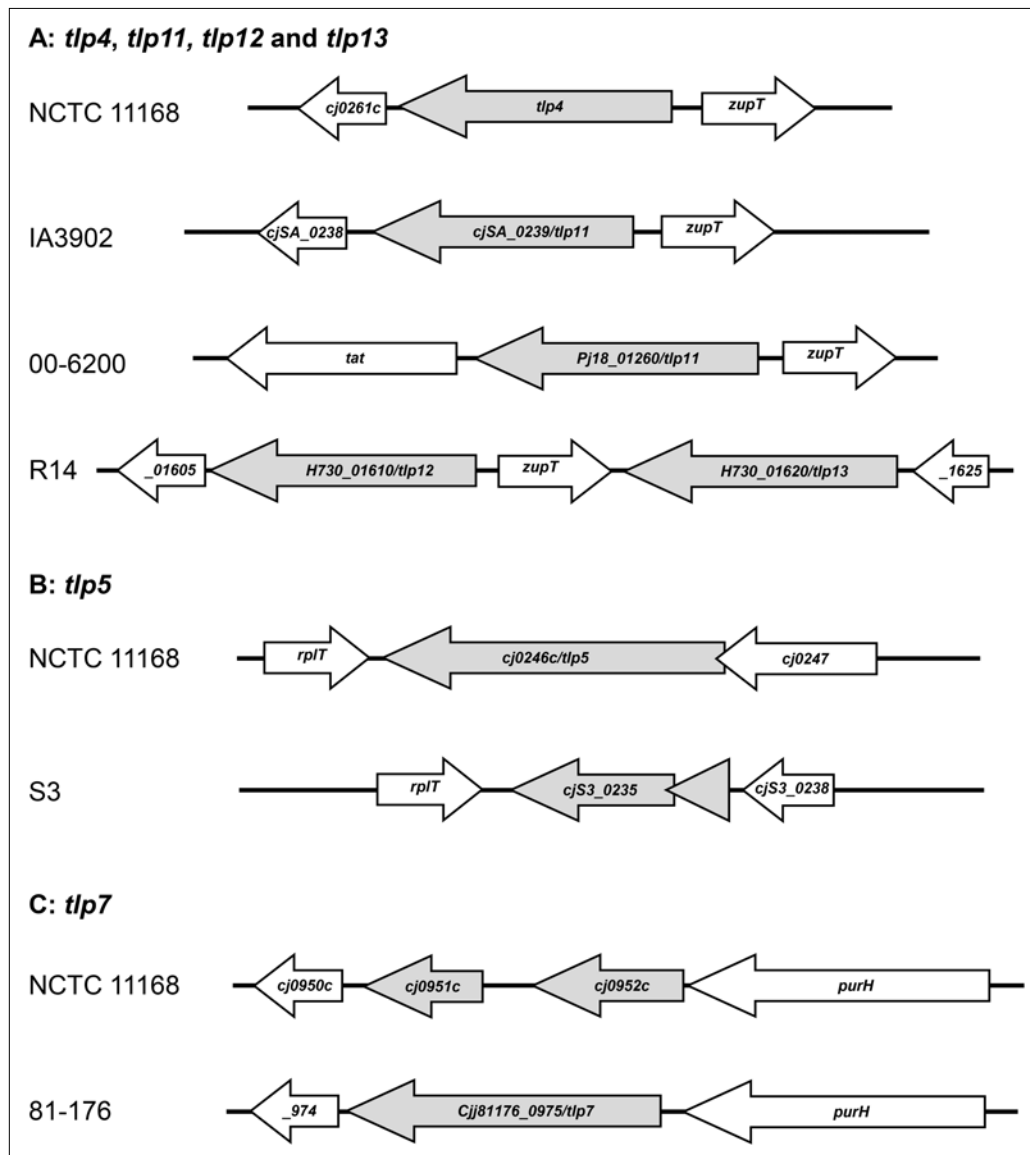
BLAST search of all 15 known chemoreceptor genes against the 142 assembled *C. jejuni* ssp. *jejuni* genome se-

quences deposited in the NCBI genome database (<http://www.ncbi.nlm.nih.gov/genome/genomes/149>) showed that the genes *tlp1*, *tlp2*, *tlp3*, *tlp6*, *tlp8*, *tlp9*, and *tlp10* were well conserved (coverage: ca. 100%; sequence identity ca. 97%). There was only limited sequence variability, and there were no pseudogenes close to the particular gene loci.

In contrast, significant variation was observed in genes *tlp4*, *tlp5*, *tlp7*, and *tlp11*.

### Gene sequence differences of *tlp4* and *tlp12*

The chemoreceptor gene *tlp4* (*cj0262c*) was 1998 bp in strain NCTC 11168. It was flanked by gene *cj0261c*



**Fig. 1.** Genome arrangements of different bacterial strains at the gene loci of *tlp4*, *tlp11*, *tlp12*, and *tlp13*; *tlp5* as well as *tlp7*. A: The *tlp4* gene is located downstream of *zupT* in strain NCTC 11168. In strain IA3902, the *tlp4* gene downstream of *zupT* is replaced by gene encoding Tlp11. A SAM-dependent methyltransferase gene downstream of the *tlp4* (NCTC 11168) or *tlp11* (IA3902) gene is replaced by transcription initiation protein gene *tat* in strain 00-6200. In strain R14, the gene encoding Tlp12 is located downstream of *zupT*. Upstream of *zupT*, a *tlp13* gene is present. B: The *tlp5* gene in NCTC 11168 *cj0246c* is replaced by a disrupted pseudogene in strain S3. C: *Tlp7* gene is interrupted by a stop codon at position 891 663 of the NCTC 11168 genome splitting the open reading frame into two parts *cj0952c* and *cj0951c*; in strain 81-176, *tlp7* is one continuous gene

encoding a S-adenosyl-L-methionine (SAM)-dependent methyltransferase and gene *zupT* encoding a zinc transporter (Fig. 1A). Between these two genes, we discovered in the genome sequence of strain R14 a chemoreceptor gene (*H730\_01610*) that was 1989 bp. The 173 bp at the 5' end and the 1134 bp at the 3' end of *H730\_01610* showed significant sequence identity to gene *tlp4* (*cj0262c*) of NCTC 11168: 91% (158/173) and 96% (1085/1136), respectively (Fig. 2). In contrast, the 682 bp in between these two largely identical sequence regions shared no significant sequence identity. Therefore, we concluded that *H730\_01610* (in strain R14) is a new receptor and named homologues to chemoreceptor gene *H730\_01610* *tlp12*. Homologues to chemoreceptor gene *cj0262c* (*tlp4* in strain NCTC 11168) were further referred to as *tlp4*. The overall sequence identity between *tlp4* and *tlp12* was 83% (1679/2023) for the DNA sequence.

#### Gene sequence differences of *tlp11* and *tlp13*

Upstream of *zupT*, we identified a further methyl-accepting chemotaxis protein gene, *H730\_01620*, in the genome of strain R14 (Fig. 1A). *H730\_01620* was 2118 bp, and the 951 bp at the 3' end encoding the cytoplasmic signaling domain was 98% (936/953) identical to the cytoplasmic signaling domain of *tlp4/cj0262c* and 94% (897/954) identical to *tlp12* (*H730\_01610*; Fig. 2). In contrast, the 3' 2004 bp of *H730\_01620* was 85% (1721/2018) identical to gene *cjj8425\_0287* of strain 84-25, which was designated as *tlp11* [10]. Interestingly, the first 114 bp of *H730\_01620* differs from *tlp11* (*cjj8425\_0287*). Therefore, we concluded that *H730\_01620* was also a new receptor gene. Consequently, we named homologues to chemoreceptor gene *H730\_01620* in R14 as *tlp13*. Homologues to chemoreceptor gene *cjj8425\_0287* in strain 84-25 were further referred to as *tlp11*. The overall sequence identity between *tlp11* and *tlp13* was 85% (1703/2006) for the DNA sequence.

However, there was some variation in the flanking regions of *tlp11*. For example, in strain IA3902, *tlp11* was located between SAM-dependent methyltransferase gene *cjSA\_0238* and zinc transporter gene *zupT*, completely replacing *tlp4*, and in strain 00/6200, *tlp11* was flanked by transcription initiation protein gene *tat* and zinc transporter gene *zupT* (Fig. 1A).

#### Multiple sequence alignment of *tlp2*, *tlp3*, *tlp4*, *tlp11*, *tlp12*, and *tlp13*

Multiple sequence alignment of *tlp4* (*cj0262c*), *tlp11* (*CJJ8425\_0287*), *tlp12* (*H730\_01610*), *tlp13* (*H730\_01620*), *tlp2* (*cj0144*), and *tlp3* (*cj1564*) indicated that *tlp4*, *tlp11*, *tlp12*, and *tlp13* form a phylogenetically related group, while *tlp2* and *tlp3* were distant and, hence, less related to *tlp4*, *tlp11*, *tlp12*, and *tlp13*.

The DNA sequence identity of *tlp12* was 50.0% (994/1989) referring to *tlp2* and 50.0% (994/1989) referring to *tlp3*.

Comparably, DNA sequence identity of *tlp13* was 44.2% (936/2118) referring to *tlp2* and 44.2% (936/2118) referring to *tlp3*. Therewith, the sequence identity was significantly lower in comparison to *tlp4* and *tlp11* (Figs. 2 and 3).

#### *Tlp5* intact gene and *tlp5* pseudogene

Intact gene *tlp5* encompassed 1128 bp in strain NCTC 11168 (*cj0246c*). It was located between gene *rplT* that encodes the L20 ribosomal protein of the 50S subunit and *cj0247* that encodes a chemotaxis sensory transducer protein. In five of the deposited genomes, namely, S3, 00-1597, F38011, CG8421, and R14, *tlp5* gene was disrupted at position 504 of the gene, and at the same gene locus, a pseudogene of about 537 bp was found (annotated as disrupted methyl-accepting chemotaxis protein [MCP]-domain signal transduction protein pseudogene, Fig. 1B). The 503 bp at the 5' end of the pseudogene shared 99% identity to the sequence of the periplasmic sensory domain of the complete *tlp5* gene, but the cytosolic signaling domain was missing.

#### Detection of chemoreceptor genes in a phylogenetically diverse *C. jejuni* strain collection using PCR

##### Ubiquitous chemoreceptor genes

Analyzing a strain collection of 292 *C. jejuni* ssp. *jejuni* isolates for the so far well-described 13 chemoreceptor genes, two aerotaxis genes, *tlp5* pseudogene, *tlp7* two ORF variant, *tlp12*, and *tlp13* using PCR, showed that a majority of the chemoreceptor genes were found to be ubiquitous. In detail, *tlp1* (*ccaA*) was detected in 100% (292/292), *tlp2* in 97.9% (286/292), *tlp3* (*ccmL*) in 100% (292/292), *tlp6* in 100% (292/292), *tlp8* in 100% (292/292), *tlp9/cetABC* (including both cytoplasmic ligand-proteins: *cetA*, *cetB/aer2*, *aer1*) in 100% (292/292), and *tlp10* in 97.9% (286/292) of the *C. jejuni* ssp. *jejuni* isolates (Fig. 4).

Six (2.1%; 6/292) *tlp2* negative isolates were detected in the MLST clonal complex (CC) CC48-related CCs, and six (2.1%; 6/292) *tlp10* negative isolates were detected in the MLST sequence type (ST) ST563, ST54, ST677, and CC45 isolates. However, these differences, in comparison to the remaining isolate population, were not significant (Table 2, Fig. 4).

##### Transducer-like protein genes *tlp4*, *tlp11*, *tlp12*, and *tlp13*

As shown in Fig. 1A, *tlp4*, *tlp11*, *tlp12*, and *tlp13* shared nearly the same gene locus downstream (*tlp4*, *tlp11*, *tlp12*) or upstream (*tlp13*) of the *zupT* gene. It seemed that these genes substitute each other at one site in the genome, but in some genomes, they were found in close proximity to each

```

tlp11_CJJ8425_0287   ATG-----AATTTTCGTTCTCTAAATTTAAG-----TACTAAGCTTATT
tlp13_H730_01620    -----ATGTTTAGACTATCGAGCGTAG-----TTCAAAACCTTTA
tlp4_cj0262c        atgcaatcaata-----aattc-aggcaaa--tccgttggaatctcagctaagcttacg
tlp12_H730_01610    ATGCAATCAATA-----AATTC-AGGCAAA--TCCGTTGGAATTCAGCCAAGCTTACG
tlp2_cj0144         -----atgaaaagcgtaaaattgaaggtttcg
tlp3_cj1564         atgctaaaaataacaagattaaaaggaaaataatgaatagattataaaatcaaaccttcc
                                     *           **   *

tlp11_CJJ8425_0287   TTATCTGTAGCAATAGGAATAGTCTTAGGCATAGTTGTAATTGTTTTAACCGTATCTATA
tlp13_H730_01620    TTATCAGTTGCTATTAGTGTATTGTAGCTATAGCTCTTATGATTGCTATTGTTTCTTTT
tlp4_cj0262c        ctatgggttggaatcttagtattgataatcttagcaatcacaagtgctatta-----
tlp12_H730_01610    CTATGGGTTGGAATTTTAGTTGTATTAATTTTAGCAATCACAAGTACCGTTA-----
tlp2_cj0144         ctgattgcaaatttaatcgcagtagtgtgtttgataatcttaggtgttgtaa-----
tlp3_cj1564         ctcatggcaaatttaattgcaatctttgccttaattgttctaggtattgtaa-----
                                     *   *           * * *   *   *

tlp11_CJJ8425_0287   TATACTTCTAAAAGTATGGAAAAAGAAGCTAAAGATTCAATATTTTTGTCTTCAAAAAGA
tlp13_H730_01620    CAAGTTGCTTCATATTCAGAAAAAGAAGCCAAAGATACTATTTTTTTTATCTTCAAAAAGA
tlp4_cj0262c        ----gttact-----ttgattcggagaacaata
tlp12_H730_01610    ----GTTACT-----TTGATGCCAAAAATCACA
tlp2_cj0144         ----cattta-----tatttgtaaagcaagcaa
tlp3_cj1564         ----gttttt-----atttacaaaaacctcac
                                     *

tlp11_CJJ8425_0287   TATGTCAATTACATGGAAGGTATTTTAAATGAAGAGGTTGTATTAACAAAAGCAATGGCC
tlp13_H730_01620    TATGTGAATTATATACAAGGTATTTTAAATGAAGAAGTCACACTAACCAAAGGAGTTGCT
tlp4_cj0262c        catatgaattgctaaaa-----gacactcagttaaaaactatgcaagatgtggat
tlp12_H730_01610    CATATGAATTGCTAAAA-----GAAAATCAATTAAAAACTATGGATGATGTTAAA
tlp2_cj0144         tttttcatgaagttgtg-----aatgctgaaataaattatgttaaaacggctaaa
tlp3_cj1564         tatatgaaagcactctt-----aaaaatcaaactgacactcttaaagtcacacaa
                                     * * *           *   *

tlp11_CJJ8425_0287   ACTTCATTAATGAAATATTTTCAAAAATGATCAAGTGAACGCTGGTATAATAGAAAAGT
tlp13_H730_01620    ACATCGTTAAATGAAATGTTTCAAAAATGATCATGTAGATATCGATTTAATGAAAAGT
tlp4_cj0262c        gctttctttaaaagctatgctatgtcaaaaagaaatggtattcaaatactagccaatgag
tlp12_H730_01610    GTAACTTTTGAAAACATTTCCAAAAGCAAGCAAAAAGCTATAGAGGTTCTAGCATATGAA
tlp2_cj0144         aattctatagagctttttaaggcaagaaattcttttagctcttgaaagtttagctaaaagt
tlp3_cj1564         tctaccgttgaagatttccggtccacaaatcaatcttttactagagctttagaaaaagat
                                     *   *           **           **   *

tlp11_CJJ8425_0287   CTTTTAAGAAATACATTTGATAGTAGCGGTTATGCAGCTTATGCTTTTCTTTATTTACAG
tlp13_H730_01620    CTTATAAAAAATACTTTTGTAGTAGTCACTATGCAGCATATACATTTCTCTATTTAAAA
tlp4_cj0262c        ctaacaaatcgtcctg-----atat-----gagc
tlp12_H730_01610    AGTGCTAAAAAAT-----T-----AGAA
tlp2_cj0144         attttaagcatcctatagaacagttaga-----tagt
tlp3_cj1564         atcgcgaacttaccttatcaatctttaat-----cact
                                     *

tlp11_CJJ8425_0287   GATTCTTCTATACTAACTCATGTTGAATCTTTAGATAAAAAATTTTAAAAACTCTGATGGC
tlp13_H730_01620    GATACTACTGTATTAAGTGATATGCAGAATGTAGATAAAAAATATATTAGCCAGATGGA
tlp4_cj0262c        gatgaagagctaatcaat---cttatcaagtaattaaa-----aaagttaa---
tlp12_H730_01610    GATGAAAATATTTCACTG---CTTCTAGATTCTTTTAAAG-----AAGGCGTT----
tlp2_cj0144         caagatgctttaatgcattatggttgaaaagatttaagaattttagagatgctgg---
tlp3_cj1564         gaagaaaatattattaacaatggttggtccaatattgaaatattatcgtcatagtat---
                                     *   *           *           **

tlp11_CJJ8425_0287   AAAAGTGAACAATGATTTTTTTTGGATGAAACCACAGGGAAGGCAGGCGGTATTTAAAGC
tlp13_H730_01620    AAAACTTTTTCTATGATATTTTTTGGATCAAATAGCAGAAAAATCTGGTGGAATAACTACT
tlp4_cj0262c        --tgactacgatctagtttatgtaggatttgataatacagga-----aaaaattat
tlp12_H730_01610    --TGATTTTGATATTGTTTTTATTGCGTTTGATAAAAAACAAC-----AAAATGCTT
tlp2_cj0144         --aagattcttagcagtttatattgctcaacaaatggcgaacttgttgtaagcgatcca

```

**Fig. 2.** Multiple sequence alignment of *tlp2* (*cj0144*), *tlp3* (*cj1564*), *tlp4* (*cj0262c*), *tlp11* (*CJJ8425\_0287*), *tlp12* (*H730\_01610*), and *tlp13* (*H730\_01620*). The multiple DNA sequence alignment was analyzed using Clustal Omega (<http://www.ebi.ac.uk/Tools/msa/clustalo/>). An asterisk indicates identical bases in all six sequences



```

tlp11_CJJ8425_0287 ATACACGCTCCGAGCAATTTTTCACAAC-----ACCTATTATAGAAAAAATT
tlp13_H730_01620 ATTTCAACTCCAAAACAATTTCTCTCAATT-----AAATCTAATACAAAATATC
tlp4_cj0262c caatctgatgatcaaatTTTTtagatct-----atcaaaaggttatgatact
tlp12_H730_01610 TTGTCAAATGGAACAATTTTAGATA-----AAAATCAAATTTTGACATT
tlp2_cj0144 gactctgatgctaaaaatTTtagatTTTTggaacttatggaaaagctgataattatgatgct
tlp3_cj1564 --atctaag-----atgcaaaaatgacctgaattacgtgatgatttagatata
* * *

tlp11_CJJ8425_0287 AAAAAAATGCAAGATATGGTGAT---CTTGATACTATATTTT TAGGATCTCCATCAAGA
tlp13_H730_01620 GAACAAAATGCAAAATATGGAGAT---AAAGACTCTGTTTTTGTAGGTTCTCCTAGAAAA
tlp4_cj0262c aaaaatcgctccttggataaaagctgccaaagaagcaaaaaagcttatagtaacagaacct
tlp12_H730_01610 ACAAACAAATTTGGTATCAAGAAGCAAAAAATAACAAAGGCATCACAATAACTCAACCA
tlp2_cj0144 agaacaagagagtattatataagaagcagttaaaacaaataaactttatattaccccatct
tlp3_cj1564 aagacaaaagattggtatcaagaagcttttaaaaacaatgatatttttggttaccaccaga
*** * * * *

tlp11_CJJ8425_0287 CTTAATTATGATGGAACAGAATTTT TAGGTATTAACCTAGGAATGCCCTTATTTAATAAA
tlp13_H730_01620 CTTAATTATGATAATAATGAATTTT TAGGTATAAATTTCCGTATGCCTATTTTAACAAT
tlp4_cj0262c tataaatccgcccgtagcggagaggttggtttaacttacgctgctccattttatgataga
tlp12_H730_01610 TATAAATCCCTATAGATCAAGAGATTGGTATAAATTATGTTTTCCCTATTTATAA---A
tlp2_cj0144 tatattgatgtaactacaaatTTaccttgctttacatattctattccgctttataa---a
tlp3_cj1564 tatttagatacagtttttaaaacaatatgtaataacgtattttaaagctatttataa---a
* * * * *

tlp11_CJJ8425_0287 GAAGGAAAATTTATCGGTATAGTTGGATTTACTTTTGATTTTTT TAGAAATATCTGAAACA
tlp13_H730_01620 AAGGGCAAATTTATGAGATTATAGGATATACTATAGATTTACTAGAAATTTCCGAGACT
tlp4_cj0262c aatggaatTTTTtagaggtggttaggtggagattatgatctagcaatTTTTcaaccaat
tlp12_H730_01610 AATAATCAACTTATAGCTTTCCGTTGGAGGCGATTACAATCTAGATAAATTTCCAAAGAT
tlp2_cj0144 gatggtaaatTTataggggttttggctgtagata-----ttcttgcggcagat
tlp3_cj1564 gatggtaaatcataggggtactgggtgctcgata-----taccatcagaagat
* * * * *

tlp11_CJJ8425_0287 ATACTTGATCCGAAGTTAGATTTTTTATAAAGATGATTTAAGATTTTTAATAACAGATCAA
tlp13_H730_01620 ATATTGGATCCAAAATTCGACTTTTTTGAAGGGGATCTAAGATTTCTTAATGAACGATCAG
tlp4_cj0262c gttttaact--gtaggaatcagacaatacctttactgaagtacttgat---tcagaa
tlp12_H730_01610 GTATTATCT--TTAGGACTCATCAACTACTTATGCTGCTGTTTATGAC----TCTGAA
tlp2_cj0144 ttgcaagct--gaatttgaa-----aat----ttacca
tlp3_cj1564 ttgcaaat--ttagttgca-----aaa----accctt
* * * *

tlp11_CJJ8425_0287 GGTGTTATTGTTATTCATAAGAACAAGATGCTATTTTTAAAACTTTACC-----TGAA
tlp13_H730_01620 GGTATTATTGCTATTCATAAAAAATAAAATGCAATTTTTAAAACTTTATT-----TGAT
tlp4_cj0262c ggaacaatactttttaatgatgaagttgctaaaataactaaca---aaaacagaattaagt
tlp12_H730_01610 GGAAGAATTATTTTTCACGAAGTGCTTGATAGAATTTTAACA---AAAACACTTTAAGC
tlp2_cj0144 ggtagaacttttgtatttgatgaagaaaataaagtatttgttctacagacaagctctt
tlp3_cj1564 ggaaatacttttttatttgatcaaaaaataaataatttgcagcaaccaataaagaatta
** * * * *

tlp11_CJJ8425_0287 ATTAATCAAGATGCATCCGTGCAATTAATTATT---GATGCTGTTAAAAATCATAAAGAT
tlp13_H730_01620 ATTAACAAAGATCAATCTGCTCAATTAATTGTT---GAGGCAGTTAAAAACCATAAAGAT
tlp4_cj0262c atcaatatcgccaatgcaatcaaaagcaaatcctgctcttattgatccaagaaccaagat
tlp12_H730_01610 GTTAATATTGCTAACGCCATAAAAGAAAACCTGAATATATAGATCCAAATAAGAGAGAT
tlp2_cj0144 ttacaaaagggttatgatatttagtgaattgca---aatcttgctaaaactaaagaggat
tlp3_cj1564 ttaaatccatccattgatcattctcctgcttcta---aatgcatataaactcaatgggtgat
* * * * *

tlp11_CJJ8425_0287 TTGATTATTGATAATTATGTTGACTTAAGCGGAAATTTAAGCTATGCAGGAGTTGCTTCA
tlp13_H730_01620 GAAATCTTAGATAATTATATTGCTCAACAGGAGATCTTAGTTATGCTAGTATCTCTTCA
tlp4_cj0262c ac----tttatttaccgctaaagatcaccaaggc--gtagattatgctgattatgtgtaat
tlp12_H730_01610 AT---TCTTTTTCTGTATTTGATGATAAAGGT--ATCAAATATGAAACAATGTGCGAT
tlp2_cj0144 cttgaacctttttagtatactagacaaaagatg--gtaatgaagatttgcgtgat-gc

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Fig. 2. (cont'd)

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tlp11_CJJ8425_0287   TTTAGTACCTTAGGAGATTTCGAGTCATTGGAGCATGGTTGTAACAGCACCTAAAAAATCT
tlp13_H730_01620    TTTAGCACTTTAGGTAATTCTAGTCATTGGAGTGTAATTGTAACAGCTCCTAAAAAATCA
tlp4_cj0262c        tctgcttttaaatcctttatcttagaatttgtacaat-----aacagaaaacaagtt
tlp12_H730_01610    ACAAGTTCCAATGGATTATATCGTATTTGTGCTGT-----AACTTTAGATAGTAAC
tlp2_cj0144         acaaaggtttctggaattatactgcttgcggttg-----agagccaatagaacaa
tlp3_cj1564         actaaagtctt---tgcttatacagcttgattac-----cgaaagcgctgatatt
                        *           ***

tlp11_CJJ8425_0287   ATTTTTCGCGCTTTATATGAATTGAATTTTATTCTAATTAGTATTGCAATTATTGTATTA
tlp13_H730_01620    GTTTTAGCCCTTTATATAAACTACAATATATTATTATTAGTGTGCTATAATAGCACTA
tlp4_cj0262c        tataccgaagctgtaattctatttttaagaaacaagttatagttggtattatagctata
tlp12_H730_01610    TATACTTCGCTGTTAATTCCATTTTAATGAAACAAGTTATAGTCGGTATTATAGCTATA
tlp2_cj0144         atagaagctccagtttataaaaattgcatttatacaaaactgcgattggtatttttacaagt
tlp3_cj1564         ataaataaacctatttataaagctgcatttattcaagccattggttgcattattgtagta
                        * * * * * * * * * *

tlp11_CJJ8425_0287   ATTGCTATTTTAATTATTTCTGTATTTTTTGTGTAATAAATATAGTAGGATCTAAACTTCCA
tlp13_H730_01620    ATTGCTATTTTGGCTGTGTGTAATTTTTTATAAGAAAATCATAGGCTCTAGAATCCCT
tlp4_cj0262c        atcatagctttaatct---tgattagatttttaatcagcagaagtcctctcccacttgca
tlp12_H730_01610    ATCATAGCTTTAATCT---TGATTAGATTTTAATCAGCAGAAGCCTCTCCCCACTTGCA
tlp2_cj0144         attattagcgtcatcc---tcctttatttcacgtatcaaaatacctctcccacttgca
tlp3_cj1564         gtatttagcgtcatcc---tcctttatttcacgtatcaaaatacctctcccacttgca
                        * * * * * * * * * *

tlp11_CJJ8425_0287   ATTATAGTTAATTCCTGCAAAATTTCTTTGACTTTTATCAACTATAAAACAAAAATGTT
tlp13_H730_01620    CTTATACTTAAGTCTTTGGAAAATTTCTTTGCTTTTAAATCATGAAAAATAGAAGTT
tlp4_cj0262c        gctatccaaacaggtttaacttcattctttgattttatcaactataaaacaaaaatggt
tlp12_H730_01610    GCTATCCAAACAGGTTTAACTTCATTCCTTTGATTTTATCAACTATAAAACAAAAATGTT
tlp2_cj0144         gctatccaaacaggtttaacttcattctttgattttatcaactataaaacaaaaatggt
tlp3_cj1564         gctatccaaacaggtttaacttcattctttgattttatcaactataaaacaaaaatggt
                        *** * * * * * * * * * *

tlp11_CJJ8425_0287   TCTACTATAGAAGTAAAAAGCAATGATGAATTTGGACAAATCTCAAATGCTATCAATGAA
tlp13_H730_01620    CAGACAATTGAAATTAAGCTAATGATGAATTTGGCAAAATGGGTAATAATATCAACGAA
tlp4_cj0262c        tccactatagaagtaaaaaagcaatgatgaatttgacaaatctcaaatgctatcaatgaa
tlp12_H730_01610    TCTACTATAGAAGTAAAAAGCAATGATGAATTTGGACAAATCTCAAATGCTATCAATGAA
tlp2_cj0144         tccactatagaagtaaaaaagcaatgatgaatttgacaaatctcaaatgctatcaatgaa
tlp3_cj1564         tccactatagaagtaaaaaagcaatgatgaatttgacaaatctcaaatgctatcaatgaa
                        ** * * * * * * * * * *

tlp11_CJJ8425_0287   AACATTCTTGCTACTAAAAGAGGCTTAGAACAAGACAATCAAGCCGTTAAAGAATCAGTT
tlp13_H730_01620    AACATTCTTGCTACTAAAAGAGGCTTAGAACAAGACAATCAAGCCGTTAAAGAATCAGTT
tlp4_cj0262c        aacattcttgctactaaaagaggcttagaacaagacaatcaagccgtaagaatcagtt
tlp12_H730_01610    AACATTCTTGCTACTAAAAGAGGCTTAGAACAAGACAATCAAGCCGTTAAAGAATCAGTT
tlp2_cj0144         aacattcttgctactaaaagaggcttagaacaagacaatcaagccgtaagaatcagtt
tlp3_cj1564         aacattcttgctactaaaagaggcttagaacaagacaatcaagccgtaagaatcagtt
                        *****

tlp11_CJJ8425_0287   CAAACCGTATCAGTTGTAGAAGGTGGTAATTTAACAGCAAGAATTACTGCTAATCCAAGA
tlp13_H730_01620    CAAACCGTATCAGTTGTAGAAGGTGGTAATTTAACAGCAAGAATTACTGCTAATCCAAGA
tlp4_cj0262c        caaacgctatcagttgtagaaggtggaatTTAACAGCAAGAATTACTGCTAATCCAAGA
tlp12_H730_01610    CAAACCGTATCAGTTGTAGAAGGTGGTAATTTAACAGCAAGAATTACTGCTAATCCAAGA
tlp2_cj0144         caaacgctatcagttgtagaaggtggaatTTAACAGCAAGAATTACTGCTAATCCAAGA
tlp3_cj1564         caaacgctatcagttgtagaaggtggaatTTAACAGCAAGAATTACTGCTAATCCAAGA
                        *****

tlp11_CJJ8425_0287   AACCCACAGCTTATTGAACTTAAAAATGTTCTAAATAAACTTCTTGATGTTTTACAGCT
tlp13_H730_01620    AACCCACAACCTGATTGAACTTAAAAATGTTCTAAATAAACTTCTTGATGTTTTACAGCT
tlp4_cj0262c        aaccacagcttattgaaacttaaaaatggttctaataaaacttcttgatgTTTTACAGCT
tlp12_H730_01610    AACCCACAATTAATAGAATTAAAAAATGTTCTAAATAAACTTCTTGATGTTTTACAGCT
tlp2_cj0144         aaccacagcttattgaaacttaaaaatggttctaataaaacttcttgatgTTTTACAGCT

```

Fig. 2. (cont'd)

tlp11_CJJ8425_0287	AGAGTAGGTTCTGATATGAATGCTATTTCATAAAAATTTTGAAGAATACAAAAGCTTAGAC
tlp13_H730_01620	AGAGTAGGTTCTGATATGAATGCTATTTCATAAAAATTTTGAAGAATACAAAAGCTTAGAC
tlp4_cj0262c	agagtaggttctgatatgaatgctatttcataaaaatTTTTgaagaatacaaaaagcttagac
tlp12_H730_01610	AGAGTAGGTTCTGATATGAATGAAATTCAAAGAGTATTTAATAGTTATAAATCTCTTGAC
tlp2_cj0144	agagtaggttctgatatgaatgctatttcataaaaatTTTTgaagaatacaaaaagcttagac
tlp3_cj1564	agagtaggttctgatatgaatgctatttcataaaaatTTTTgaagaatacaaaaagcttagac ***** *
tlp11_CJJ8425_0287	TTTAGAAATAAATTAGAAAATGCTAGCGGTAGGTGAGAATTAACACTAATGCTTTAGGT
tlp13_H730_01620	TTTAGAAATAAATTAGAAAATGCTAGCGGTAGGTGAGAATTAACACTAATGCTTTAGGT
tlp4_cj0262c	tttagaaataaattagaaaatgctagcggtagtgtagaattaactactaatgcttttaggt
tlp12_H730_01610	TTTACTACTGAAGTAAAAGATGCCAATGGAGCTGTAGAGGTAACACTAATGCCTAGGA
tlp2_cj0144	tttagaaataaattagaaaatgctagcggtagtgtagaattaactactaatgcttttaggt
tlp3_cj1564	tttagaaataaattagaaaatgctagcggtagtgtagaattaactactaatgcttttaggt **** *
tlp11_CJJ8425_0287	GATGAAATAGTTAAAATGCTAAAACAAAGTTCAGACTTTGCCTAATGCTTTAGCTAATGAA
tlp13_H730_01620	AATGAAATAGTTAAAATGCTAAAACAAAGTTCAGACTTTGCCTAATGCTTTAGCTAATGAA
tlp4_cj0262c	gatgaaatagttaaaatgctaaaacaaagttcagacttttgctaatgcttttagctaata
tlp12_H730_01610	CAAGAAATCATTAAAATGCTAAAACAAAGTTCAGACTTTGCCTAATGCTTTAGCTAATGAA
tlp2_cj0144	gatgaaatagttaaaatgctaaaacaaagttcagacttttgctaatgcttttagctaata
tlp3_cj1564	gatgaaatagttaaaatgctaaaacaaagttcagacttttgctaatgcttttagctaata *
tlp11_CJJ8425_0287	AGTGGAAAATACAACTGCTGTTCAAAGCTTAACCACTTCTTCAAATTCCTCAAGCTCAA
tlp13_H730_01620	AGTGGAAAATACAACTGCTGTTCAAAGCTTAACCACTTCTTCAAATTCCTCAAGCTCAA
tlp4_cj0262c	agtggaaaattacaactgctgttcaaagcttaaccacttcttcaaattctcaagctcaa
tlp12_H730_01610	AGTGGAAAATACAACTGCTGTTCAAAGCTTAACCACTTCTTCAAATTCCTCAAGCTCAA
tlp2_cj0144	agtggaaaattacaactgctgttcaaagcttaaccacttcttcaaattctcaagctcaa
tlp3_cj1564	agtggaaaattacaactgctgttcaaagcttaaccacttcttcaaattctcaagctcaa *****
tlp11_CJJ8425_0287	TCTTTAGAAGAACTGCAGCAGCTTTAGAAGAGATCACTTCTTCTATGCAAATGTTTCA
tlp13_H730_01620	TCTTTAGAAGAACTGCAGCAGCTTTAGAAGAGATCACTTCTTCTATGCAAATGTTTCA
tlp4_cj0262c	tctttagaagaactgcagcagcttttagaagagatcacttcttctatgcaaaatgtttca
tlp12_H730_01610	TCTTTAGAAGAACTGCAGCAGCTTTAGAAGAGATCACTTCTTCTATGCAAATGTTTCA
tlp2_cj0144	tctttagaagaactgcagcagcttttagaagagatcacttcttctatgcaaaatgtttca
tlp3_cj1564	tctttagaagaactgcagcagcttttagaagagatcacttcttctatgcaaaatgtttca *****
tlp11_CJJ8425_0287	GTTAAAACACTAGTGTGTTATCACTCAATCCGAAGAGATTAATAATGTTACAGGTATTATA
tlp13_H730_01620	GTTAAAACACTAGTGTGTTATCACTCAATCCGAAGAGATTAATAATGTTACAGGTATTATA
tlp4_cj0262c	gttaaaactagtgtattatcactcaatctgaagagattaaaatgttacaggtattata
tlp12_H730_01610	GTTAAAACACTAGTGTGTTATCACTCAATCCGAAGAGATTAATAATGTTACAGGTATTATA
tlp2_cj0144	gttaaaactagtgtattatcactcaatctgaagagattaaaatgttacaggtattata
tlp3_cj1564	gttaaaactagtgtattatcactcaatctgaagagattaaaatgttacaggtattata *****
tlp11_CJJ8425_0287	GGTGATATTGCAGATCAAATCAATCTTTTGTAGCTTTAAATGCAGCTATTGAAGCAGCTCGT
tlp13_H730_01620	GGTGATATTGCAGATCAAATCAATCTTTTGTAGCTTTAAATGCAGCTATTGAAGCAGCTCGT
tlp4_cj0262c	ggtgatattgcagatcaaatacaatcttttagctttaaatgcagctattgaagcagctcgt
tlp12_H730_01610	GGTGATATTGCAGATCAAATCAATCTTTTGTAGCTTTAAATGCAGCTATTGAAGCAGCTCGT
tlp2_cj0144	ggtgatattgcagatcaaatacaatcttttagctttaaatgcagctattgaagcagctcgt
tlp3_cj1564	ggtgatattgcagatcaaatacaatcttttagctttaaatgcagctattgaagcagctcgt *****
tlp11_CJJ8425_0287	GCTGGAGAACATGGTAGAGGCTTTGCAGTGGTAGCTGATGAAGTTAGAAAGTTAGCTGAA
tlp13_H730_01620	GCTGGAGAACATGGTAGAGGCTTTGCAGTGGTAGCTGATGAAGTTAGAAAGTTAGCTGAA
tlp4_cj0262c	gctggagaacatggttagaggctttgcagtggttagctgatgaagttagaaagtttagctgaa
tlp12_H730_01610	GCTGGAGAACATGGTAGAGGCTTTGCAGTGGTAGCTGATGAAGTTAGAAAGTTAGCTGAA
tlp2_cj0144	gctggagaacatggttagaggctttgcagtggttagctgatgaagttagaaagtttagctgaa

Fig. 2. (cont'd)

tlp3_cj1564	gctggagaacatggtagaggctttgcagtggttagctgatgaagttagaagttagctgaa *****
tlp11_CJJ8425_0287	AGAACTCAAAAGTCTTTATCTGAAATTGAAGCTAATACTAATTTACTTGTTCATCTATC
tlp13_H730_01620	AGAACTCAAAAGTCTTTATCAGAAATTGAAGCTAATACTAATTTACTTGTTCATCTATC
tlp4_cj0262c	agaactcaaaagtctttatcagaaattgaagctaataactaatttacttgttcaatctatc
tlp12_H730_01610	AGAACTCAAAAGTCTTTATCAGAAATTGAAGCTAATACTAATTTACTTGTTCATCTATC
tlp2_cj0144	agaactcaaaagtctttatcagaaattgaagctaataactaatttacttgttcaatctatc
tlp3_cj1564	agaactcaaaagtctttatcagaaattgaagctaataactaatttacttgttcaatctatc *****
tlp11_CJJ8425_0287	AATGATATGGCAGAAAGTATTAAAGAACAAACTGCAGGTATCACTCAAATCAATGATAGC
tlp13_H730_01620	AATGATATGGCAGAAAGTATTAAAGAACAAACTGCAGGTATCACTCAAATCAATGATAGC
tlp4_cj0262c	aatgatatggcagaaagtattaaagaacaaactgcaggtatcactcaaatcaatgatagc
tlp12_H730_01610	AATGATATGGCAGAAAGTATTAAAGAACAAACTGCAGGTATCACTCAAATCAATGATAGC
tlp2_cj0144	aatgatatggcagaaagtattaaagaacaaactgcaggtatcactcaaatcaatgatagc
tlp3_cj1564	aatgatatggcagaaagtattaaagaacaaactgcaggtatcactcaaatcaatgatagc *****
tlp11_CJJ8425_0287	GTAGCTCAAATTGATCAAACACTACTAAAGATAATGTTGAAATTGCTAATGAATCAGCTATT
tlp13_H730_01620	GTAGCTCAAATTGATCAAACACTACTAAAGATAATGTTGAAATTGCTAATGAATCAGCTATT
tlp4_cj0262c	gtagctcaaatgatcaaactactaaagataatggtgaaattgctaataatgaatcagctatt
tlp12_H730_01610	GTAGCTCAAATTGATCAAACACTACTAAAGATAATGTTGAAATTGCTAATGAATCAGCTATT
tlp2_cj0144	gtagctcaaatgatcaaactactaaagataatggtgaaattgctaataatgaatcagctatt
tlp3_cj1564	gtagctcaaatgatcaaactactaaagataatggtgaaattgctaataatgaatcagctatt *****
tlp11_CJJ8425_0287	ATTTCTAGTACAGTAAGTGATATAGCTAATAATATCTTAGAAGATGTTAAGAAGAAGAGG
tlp13_H730_01620	ATTTCTAGTACAGTAAGTGATATAGCTAATAATATCTTAGAAGATGTTAAGAAGAAAAGG
tlp4_cj0262c	atctctagtagcagtaagtgatataagctaaataatcttagaagatgtaagaagaagagg
tlp12_H730_01610	ATTTCTAGTACAGTAAGTGATATAGCTAATAATATCTTAGAAGATGTTAAGAAGAAAAGG
tlp2_cj0144	atctctagtagcagtaagtgatataagctaaataatcttagaagatgtaagaagaagagg
tlp3_cj1564	atctctagtagcagtaagtgatataagctaaataatcttagaagatgtaagaagaagagg ***** **
tlp11_CJJ8425_0287	TTTTAA
tlp13_H730_01620	TTTTAA
tlp4_cj0262c	ttttaa
tlp12_H730_01610	TTTTAA
tlp2_cj0144	ttttaa
tlp3_cj1564	ttttaa *****

Fig. 2. (cont'd)

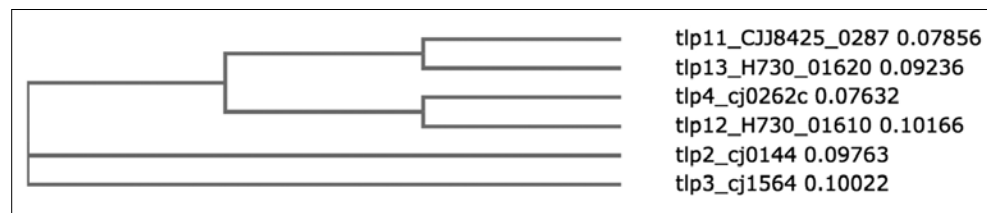
other (Fig. 1A, e.g., R14). Therefore, *tlp4*, *tlp11*, *tlp12*, and *tlp13* have to be evaluated conjointly.

***Tlp4*:** In 33.6% (98/292) of the isolates, the chemoreceptor gene *tlp4* was detected. As shown in Table 2 and Fig. 4, *tlp4*-positive isolates were predominantly present in eight clusters of isolates, namely, CC52, CC49, CC206 (ST122 and ST572), CC48 (ST48), CC22, CC45, CC283, and CC42 (Table 2). Noticeably, *tlp4* was significantly ( $p < 0.001$ ) less often detectable in turkey isolates, i.e., 8.3% (2/24).

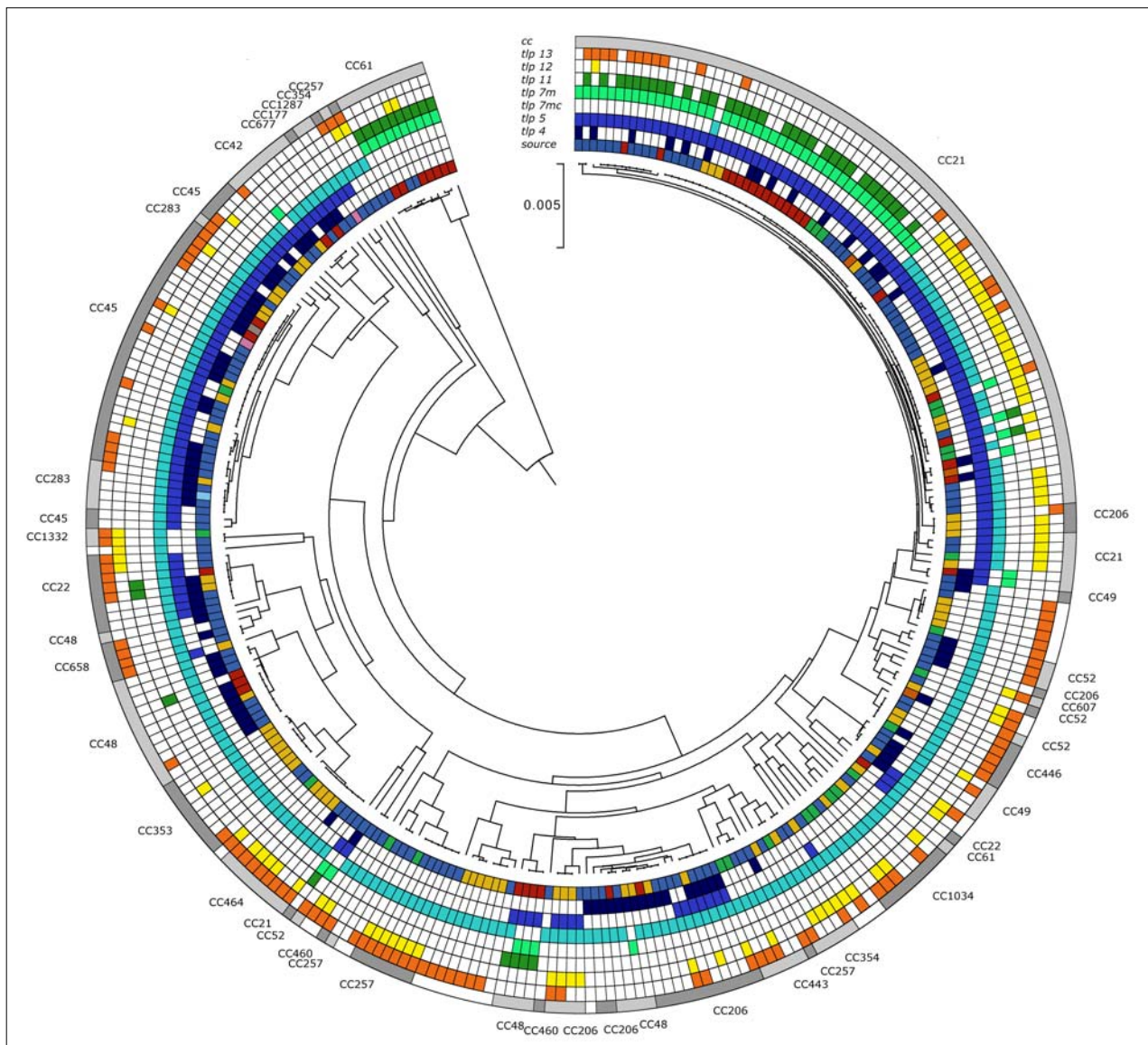
***Tlp11*:** A percentage of 17.8% (52/292) of all isolates were *tlp11* positive. Typical *tlp11*-positive isolate clusters were CC21 (ST21 and ST53), CC48 (ST38), and CC61 (Table 2, Fig. 4). Noticeably, *tlp11* was significantly ( $p < 0.001$ ) more often detected in bovine isolates, i.e., 55.8% (24/43). Only 1.7% (5/292) of the isolates were *tlp4* and *tlp11* positive.

***Tlp12*:** The PCR detecting chemoreceptor gene *tlp12* was positive in 29.5% (86/292) of the isolates. In contrast to *tlp4*, *tlp12* was predominantly present in the clonal complexes/sequence types: ST50, CC354, ST2192, CC257, ST464, and CC1332 (Table 2, Fig. 4). In the above-named CCs/STs positive for *tlp4* or *tlp11*, *tlp12* was significantly less detected (9.4%; 9/96). Only 2.1% (6/292) of the isolates were *tlp4* and *tlp12* positive, and only 0.7% (2/292) of the isolates were *tlp11* and *tlp12* positive. Remarkably, *tlp12* was significantly less often detected ( $p < 0.05$ ) in bovine isolates, i.e., 11.6% (5/43).

***Tlp13*:** *Tlp13* was detected in 38.7% (113/292) of all isolates. It was significantly more often observed in ST53, CC52, CC446, CC257, ST464, CC658, CC22, and CC1332 (Table 2, Fig. 4). Bovine isolates were significantly ( $p < 0.001$ ) less often positive for *tlp13* (4.7%). Furthermore, 12.9% (35/292) of all isolates, which corresponded



**Fig. 3.** Phylogenetic tree based on the multiple sequence alignment of *tlp2* (*cj0144*), *tlp3* (*cj1564*), *tlp4* (*cj0262c*), *tlp11* (*CJJ8425\_0287*), *tlp12* (*H730\_01610*), and *tlp13* (*H730\_01620*). Depicted is a neighbor-joining tree without distance corrections (<http://www.ebi.ac.uk/Tools/msa/clustalo/>). At the end of each branch, the designation of each aligned gene and the corresponding distance value is given. The phylogenetic tree indicates that *tlp4*, *tlp11*, *tlp12*, and *tlp13* are more closely related than *tlp2* and *tlp3*. Therefore, it can be assumed that these genes are paralogues, which may have arisen from an ancestral *tlp* gene



**Fig. 4.** Circular depiction of the MLST-based UPGMA tree of all tested 292 *C. jejuni* ssp. *jejuni* isolates and the distribution of non-ubiquitous *C. jejuni* ssp. *jejuni* chemoreceptor genes. This figure shows a balanced MLST-based UPGMA tree of all tested 292 *C. jejuni* ssp. *jejuni* isolates. The innermost circle indicates the isolate source: human isolates = blue, chicken isolates = yellow, bovine isolates = red, turkey isolates = green, ovine isolates = orange, wild bird isolates = pink, riparian isolates = light blue, and canine isolates = laurel green. The superimposed circles represent a particular non-ubiquitous transducer-like protein encoding gene (*tlp*). These are arranged in numerical order including *tlp4* (dark blue), *tlp5* (medium blue), *tlp7* uninterrupted gene (turquoise), *tlp7* two ORF variant (bright green), *tlp11* (dark green), *tlp12* (yellow), and *tlp13* (orange). Colored fields represent a chemoreceptor gene present in a given isolate, and white fields represent its absence (in the case of *tlp5*, the occurrence of *tlp5* pseudogene is indicated by a white field). The outermost circle indicates the clonal complex (CC). Different CCs listed in Table 2 are displayed alternately in light and dark gray. White fields in this circle indicate singletons or STs that are currently not assigned to a CC

to 31.0% (35/113) of the *tlp13* positive isolates, were positive for the *tlp13* gene but negative for *tlp4*, *tlp11*, or *tlp12*. Moreover, 14.4% (42/292) of all isolates were positive for both *tlp12* and *tlp13*, but not *tlp4* and *tlp11*. These isolates were especially found in ST257 and ST464. In 9.9% (29/292), the *tlp13* gene cooccurs with *tlp4*, especially in CC45. Only 3.4% (10/292) were positive for both *tlp11* and *tlp13* (especially ST53 isolates).

Noticeably, 11.3% (33/292) of the isolates were neither positive for *tlp4*, *tlp11*, *tlp12*, nor for *tlp13* (especially found in CC353 and CC45), whereas 2.4% (7/292) were positive for three genes of the *tlp4*–*tlp11*–*tlp12*–*tlp13* group. None of the isolates was positive for all four genes of the *tlp4/11* group.

#### Transducer-like protein gene *tlp5*

In addition to the intact *tlp5* gene, a much shorter pseudogene that lacks the cytoplasmic signaling domain was demonstrated. *Tlp5*-PCR primers used in this study were designed to detect the *tlp5* gene and the disrupted (pseudo-)gene. All tested isolates were either positive for one of these genes. 56.6% (165/292) of all tested isolates were positive for intact *tlp5*, and 43.4% (127/292) were positive for the pseudogene. Intact *tlp5*-positive isolates belonged predominantly to CC21, ST46, ST122, ST2192, ST38, CC22, CC45, CC283, and CC42 (Table 2). Additionally, it was significantly ( $p < 0.05$ ) associated with bovine isolate origin (Table 2).

#### Transducer-like protein gene *tlp7*

*Tlp7* was ubiquitous but occurred in a one-protein (58 kDa) and a heterodimeric two-protein variant (25 kDa + 33 kDa) as a result of an interrupting stop codon splitting the ORF into two (Fig. 1C). The two ORF variant was found in 22.9% (67/292) of the isolates, mainly, in isolates belonging to ST21, ST53, CC48, and CC61.

Distribution of the *tlp7* two ORF variant and *tlp11* in the isolate collection reveals a correlative relation. According to our data, 71.6% (48/67) of *tlp7<sub>m</sub>* positive isolates were *tlp11* positive. Further, 92.3% (48/52) of *tlp11* positive isolates were positive for *tlp7<sub>m</sub>*. As shown in Fig. 4, most *tlp7<sub>m</sub>* and *tlp11* positive isolates belong to ST21, ST53, CC48, and CC61.

The cooccurrence of both chemoreceptor genes *tlp7<sub>m</sub>* and *tlp11* correlated significantly ( $p < 0.001$ ) with bovine isolate origin. 62.8% (27/43) of the bovine isolates were tested positive for *tlp7<sub>m</sub>* and 55.8% (24/43) for *tlp11*. Accordingly, 51.2% (22/43) of the bovine isolates were tested positive for both *tlp7<sub>m</sub>* and *tlp11*.

In contrast, both chemoreceptor genes were significantly ( $p < 0.001$ ) less detected in isolates of chicken origin. Only 5.9% (4/68) of the isolates of chicken origin were tested positive for *tlp7<sub>m</sub>*, 5.9% (4/68) for *tlp11*, and 2.9% (2/68) contained both *tlp7<sub>m</sub>* and *tlp11*.

Generally, cooccurrence of *tlp7<sub>m</sub>* (and therewith *tlp11*) with *tlp4* and *tlp12* was rare. Only 6.8% (20/292) of the

isolates were tested positive for the cooccurrence of both *tlp7<sub>m</sub>* and *tlp4*. Similarly, only 2.1% (6/292) were tested positive for the cooccurrence of both *tlp7<sub>m</sub>* and *tlp12*.

## Discussion

Data obtained in our study revealed that most *C. jejuni* ssp. *jejuni* chemoreceptors were ubiquitous regardless of host, source of isolation, and clonal complex. This finding expands on a similar observation that was made in a previous exploratory study by Day and coworkers, which investigated the occurrence of group A receptor genes in 13 human isolates, seven chicken isolates, and 13 laboratory-maintained reference strains [10]. Day and colleagues revealed the following: ubiquitous occurrence of *tlp1* (*ccaA*); a higher but non-ubiquitous occurrence of *tlp2*, *tlp3* (*ccmL*), *tlp4*, *tlp7*, and *tlp10*; and the rare occurrence of *tlp11*. However, they did not consider association to factors such as MLST CC/ST; other hosts beyond chicken and human; group B and C receptor genes; genetic variants of *tlp7*, *tlp4*, and *tlp11*, *tlp5* pseudogene; and mutual cooccurrence of the receptors. Also, the sample size of the isolate collection that was tested was too low for authoritatively deducing conclusions about distribution of *C. jejuni* ssp. *jejuni* chemoreceptors.

Data obtained in this study reveals an absolute ubiquitous occurrence of *tlp1* (*ccaA*) and *tlp3* (*ccmL*) and a near absolute ubiquitous occurrence of *tlp2* (present in 97.3% of all tested isolates) and *tlp10* (present in 97.9% of all tested isolates). The unexpected existence of near absolute *tlp2* and *tlp10* can arise from two scenarios. First, it may be attributed to the primers used in this study. The primers were designed to bind the consensus sequences of the genome-sequenced strains of the NCBI database. Therefore, it is likely that the primer binding sites of *tlp2* and *tlp10* negative tested isolates have undergone mutations, which may yield a negative test result though the gene is present. Second, the difference could be due to real absence of the genes in the negative tested isolates; hence, further studies should be carried out to understand if and how the functions of these possibly missing chemoreceptors are compensated, e.g., during host colonization.

Interestingly, our results show that the genomic region neighboring the *zupT* gene was the most variable region regarding the chemoreceptor genes. As shown in Fig. 1A, we found four different chemoreceptor genes in this region: *tlp4*, *tlp11*, *tlp12*, and *tlp13*. Because of their level of divergence and occurrence in the genomic region neighboring the *zupT* gene, we assume that these genes are paralogues, which may have arisen from an ancestral *tlp* gene as a response to niche adaptation. It should be noted that *tlp12* and *tlp13* have not yet been described outside of this study and their function remains uncharacterized. However, there were some isolates which were negative for all four of these receptor genes, indicating that they may not be crucial for the survival of *C. jejuni* ssp. *jejuni*.

Otherwise, we detected isolate groups that were positive for single genes or a combination of two or three of these four chemoreceptors.

The gene encoding *tlp4* was detected in only 33.6% of all isolates and, hence, non-ubiquitous. A clear reason for limited availability cannot be deduced because the function and chemoeffectors of *tlp4* remain unresolved. Importantly, *tlp4*-positive isolates were limited to eight clonal complexes vis-à-vis CC22, CC42, CC45, CC283 CC48, CC49, CC206, and CC52.

Similarly, *tlp11* was detected in a minority, i.e., 17.8%, of the tested isolates. The *tlp11*-positive isolates were predominantly found in three clonal complexes: CC21 (ST21 and ST53), CC48 (ST38), and CC61, which have been found to be the major cause of campylobacteriosis in man [14]. Interestingly, 55.8% of these isolates originate from the bovine host; hence, *tlp11* could be a marker of bovine-associated strains. Another interesting observation which we found was the cooccurrence of *tlp11* and the two ORF variant of *tlp7*. Data analysis showed that 92.3% (48/52) of all *tlp11* positive isolates were positive for the two ORF variant of *tlp7* gene. The biological significance of this cooccurrence remains unclear since function and chemoeffectors of Tlp11 are unknown.

The new described chemoreceptor gene *tlp12* was present in 29.5% of all tested isolates. In particular, isolates of ST50 were positive for *tlp12*. Due to the significant differences in the amino acid sequence of the sensory domain, it seems likely that there are functional differences of Tlp12 compared to Tlp4. Further studies are required to address this issue and to identify specific chemoeffectors.

The second new representative in the group A chemoreceptor group is *tlp13* that was present in 38.7% of the isolates. The homology between *tlp11* and *tlp13* was much higher than between *tlp4* and *tlp12*; functional differences due to variations in the sensory domain between Tlp11 and Tlp13 cannot be excluded. This question must also be answered by future experiments.

A more global view on the *tlp4*, *tlp11*, *tlp12*, and *tlp13* receptor distribution gives the impression that the individual receptors of this group were mutually exclusive to a certain degree.

*Tlp9/cetA*, *aer1*, and *aer2* chemoreceptor genes of group B were ubiquitous in the entire test population. Similarly, group C receptor genes *tlp6* and *tlp8* were also ubiquitous in the surveyed isolates. This observation was attributed to the biological role that chemoreceptors of group B and C jointly play. For example, a recent study that evaluated the energy taxis system of *C. jejuni* ssp. *jejuni* established that *C. jejuni* ssp. *jejuni* is endowed with two energy taxis subsystems, namely, 1) CetABC (CetA = *tlp9*, CetB = *Aer2* and CetC = *Aer1*) and 2) CetZ = *Tlp8* [17]. This energy taxis subsystem controls *C. jejuni* ssp. *jejuni* taxis in a well-coordinated manner, and also, the weakness or failure of one component is complemented by another. The only non-ubiquitous exception in the group C receptors is *tlp5* that occurs as intact gene and as disrupted pseudogene. The occurrence of the disrupted pseudogene

in 43.5% of the tested isolates shows that Tlp5 is not crucial for survival of *C. jejuni* ssp. *jejuni* in some ecological niches. However, it is significant that the intact *tlp5* is more common in bovine isolates.

In conclusion, this study has shown that the chemoreceptor genes *tlp1*, *tlp2*, *tlp3*, *tlp6*, *tlp8*, *tlp9/cetA*, *aer1*, *aer2*, and *tlp10* are ubiquitous. *Tlp4*, *tlp11*, *tlp12*, and *tlp13* are non-ubiquitous; their occurrence is mutually exclusive (Fig. 4), and their distribution is related to specific MLST CCs/STs. Similarly, *tlp5* and its pseudogene present a mutually exclusive occurrence and an association to specific isolate groups. The findings of this study complete the picture of the complex chemoreceptor system of *C. jejuni*, which controls its flagellar driven taxis towards niches of favorable growth conditions while competing with the host's microbiota.

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## Conflicts of interest

All authors declare no conflicts of interests.

## Authors' contributions

A.E.Z. conceived the study idea, performed all mathematical analysis, and drafted the article. N.L.A.M., A.M.G., and W.O.M. performed bacterial culture, DNA isolation and PCR analysis, and MLST-PCR. R.L. performed DNA sequencing and assisted in drafting the article. U.G. participated in the study design and helped in drafting the article. All authors read, corrected, and approved the article.

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