A Novel *Campylobacter jejuni* Two-Component Regulatory System Important for Temperature-Dependent Growth and Colonization

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*Campylobacter jejuni* colonizes the intestines of domestic and wild animals and is a common cause of human diarrheal disease. We identified a two-component regulatory system, designated the RacR-RacS (reduced ability to colonize) system, that is involved in a temperature-dependent signalling pathway. A mutation of the response regulator gene racR reduced the organism’s ability to colonize the chicken intestinal tract and resulted in temperature-dependent changes in its protein profile and growth characteristics.

*Campylobacter jejuni* is the most common bacterial cause of food-borne gastroenteritis (18). The bacterium asymptotically colonizes the intestinal tracts of many animals used for food (1, 11). Contaminated meat, especially poultry, is a major source of *C. jejuni* infection. Motility is the major factor that has been directly implicated (38) in intestinal colonization. Understanding the processes involved in the colonization of the avian gut is a priority for the development of intervention strategies to control transmission.

Colonization is a multifactorial process involving adaptation by the bacterium to different microenvironments in the intestine (31). Two-component regulatory (TCR) systems are commonly used by bacteria to respond to specific environmental signals. These systems depend on two families of proteins, the sensory histidine kinases and response regulators (RR), which cooperate to transmit environmental signals to the bacterial response machinery (16). TCR systems are of particular importance for the regulation of gene expression in some bacterial species. *Helicobacter pylori*, for example, utilizes a reduced number of global regulatory proteins compared to the number *Escherichia coli* uses, and TCR systems seem to be a fundamental strategy for bacteria to respond to environmental pressures. In this report, we describe a novel TCR system important for the growth and possibly survival of *C. jejuni* in its natural intestinal habitat.

**Bacterial strains, plasmids, growth characteristics, and general methods.** *E. coli* XL1-Blue (6) was cultivated aerobically in Luria-Bertani medium (28). *C. jejuni* 81116 (NCTC 11828) (23) was cultivated in Mueller-Hinton (MH) broth and agar (Oxoid) or campylobacter blood-free selective agar (Oxoid) at 37 or 42°C under microaerobic conditions (6% hydrogen, 5% carbon dioxide, 5% oxygen, and 84% nitrogen). Where appropriate, growth medium was supplemented with kanamycin (50 μg/ml) or ampicillin (200 μg/ml). DNA manipulation was carried out as described by Sambrook et al. (28). A nested deletion kit was used to sequence pALB3 according to the manufacturer’s instructions (Pharmacia Biotech). The sequencing reaction mixtures were prepared with a *Tag* DyeDeoxy Terminator Cycle sequencing kit and were analyzed on an automated DNA sequencer. Sequence data was processed with the Wisconsin Molecular Biology software package (version 8, September 1994) from the Genetics Computer Group. The chicken colonization assay was performed as described previously (38). Two-dimensional (2-D) electrophoresis was carried out by using 20 μg of protein with Immobiline DryStrip (Pharmacia) isoelectric focusing in the first dimension (precast Immobiline DryStrip polyacrylamide gel; 180 mm; pH 3 to 10) and ExcelGel (Pharmacia) in the second dimension (precast ExcelGel sodium dodecyl sulfate-polyacrylamide gels; 245 by 180 mm; 12 to 14%). Protein bands were visualized by silver staining according to the manufacturer’s instructions. N-terminal sequencing was carried out at the Protein and Nucleic Acid Chemistry Laboratory, University of Leicester, with standard Edman degradation.

**Cloning and sequencing a response regulator gene.** A *C. jejuni* F132 genomic library in λZAP II (19) was probed with a DNA fragment isolated by PCR with degenerate primers (41) designed to amplify RR gene family members. A 1.5-kb *XbaI*/*HindIII* fragment containing the target sequence was subcloned into pUC19 to generate pALB3 (data not shown). The subcloned fragment was sequenced in both directions, showing the presence of one complete open reading frame (orf1) and the 189 bp of a second open reading frame corresponding to the N terminus (orf2). The 671-bp-long orf1 encodes a predicted protein with an Mr of 24,500, and it includes a DNA sequence identical to that of the 320-bp RR probe. Comparisons of deduced amino acid sequences by FASTA (25) and BLASTP (3) revealed an extensive degree of identity (33 to 38%) between Orf1 and known RR proteins (GenEMBL and SwissProt) and closest similarity between them.
mutagenesis (40) (primers 5′ ACA ATC ATA GG 3′). The DNA sequence. sequence data was used for the remaining unknown (28a) showed 100% nucleotide identity, and therefore, genome data were available for the unknown orf2 3′ DNA sequence.

Given that orf1 and orf2 are part of one operon, it is probable that Orf2 is the cognate sensory kinase of the Orf1 RR. The RR (Orf1) has been named RacR, and the histidine kinase (Orf2) has been named RacS (see below). The RR protein CheY (42) plays a role in the posttranslational regulation of chemotaxis, but RacR-RacS is the first full transcriptional TCR system described for C. jejuni.

Construction of a mutant by insertional inactivation. An insertional mutation was constructed in orf1 by inverse PCR mutagenesis (40) (primers 5′ GAA GAT CTT TAC CTG 3′ and 5′ GAA GAT CTA AAT CAG ACA ATC ATA GG 3′) and the subsequent insertion of a kanamycin resistance gene (34). The new construct, pALB5, was transferred into C. jejuni 81116 by electroporation (39). Two kanamycin-resistant mutants, designated AB1 and AB2, were isolated in separate electrotransformations. PCR and Southern hybridization confirmed that the mutants resulted from the allelic replacement of the wild-type RR gene by the insertionally mutated copy (data not shown). 2-D protein profiles (see Table 1) showed the absence of an approximately 25-kDa protein in both AB1 and AB2 compared to parent’s protein profile. N-terminal sequencing established this 25-kDa protein to be RacR, confirming that both mutants no longer express RacR.

Comparison between the parent’s and mutants’ growth profiles. The mutant strains form smaller colonies than the wild-type strain on either MH agar or campylobacter blood-free selective agar at both 37 and 42°C (data not shown). The growth characteristics of AB1 and AB2 were compared with those of 81116 in MH broth. When grown at 37°C, the mutants showed a growth rate similar to that of the parent strain, but they entered stationary phase earlier (Fig. 1). However, at 42°C, the optimum growth temperature of C. jejuni, the mutants showed a growth rate that was lower than that of 81116 (Fig. 1). At both temperatures, the mutants did not achieve the parental level of cell density. Therefore, RacR affects growth in vitro in a temperature-dependent manner.

Colonization of chickens. The mutant AB1 was tested in a chicken colonization model (38). Doses between 10⁶ and 10⁷ CFU were administered orally to groups of 10 1-day-old chicks housed in isolators. Colonization was evaluated by the level of colonization (number of viable bacteria recovered per gram of cecal contents [Fig. 2A]) and by the frequency of colonization (percentage of chicks colonized per dose group [Fig. 2B]). Recovered bacteria were checked by restriction fragment length polymorphism of flaAB and proven to be typical 81116-like bacteria (20) (data not shown). The mutant did not colonize the chicken intestine as well as the parent strain. The maximum level of colonization (Fig. 2A) observed with AB1 was approximately 10²-fold lower than that of 81116, and only doses of AB1 above 10⁶ CFU resulted in 100% colonization (Fig. 2B), whereas doses of wild-type 81116 above 10⁵ CFU colonized all inoculated chicks.

The inactivation of racR reduced the ability of the organism to colonize the alimentary tract in chickens. The mutant phenotype did not arise from changes in motility, as determined by both dark-field analysis and on swarm plates (data not shown). In Salmonella spp., a reduction in the ability to colonize chickens correlated with changes in the lipopolysaccharide profile (8, 35); however, no differences were observed between the lipopolysaccharide profile of 81116 and that of the racR mutant (data not shown). In the case of the racR mutant, it is likely that the reduced growth rate at 42°C (Fig. 1), as well as its inability to achieve the parental level of cell density, contrib-
utes to poor colonization. It is possible, however, that other *racR*-dependent gene products are important for colonization.

**Protein profile of the mutants.** Protein profiles at 37 and 42°C of the wild type and mutants (AB1 and AB2) were compared by 2-D gel electrophoresis (summarized in Table 1). At least 11 differentially expressed proteins were identified as members of the RacR regulon, and these proteins could be grouped (Table 1) according to the effect of the RacR mutation on expression. Three resolved protein spots were sequenced. Protein 1 (Table 1) was identified as RacR, and proteins 9 and 10 corresponded to two isoforms of a cytochrome *c* peroxidase homolog.

As observed with other RRs (2, 4, 5, 14, 26), RacR acts both as a transcriptional activator (group I) and as a repressor (group II). Group III proteins appear to be subject to thermoregulation, being absent from the parent strain only at 42°C. The disruption of *racR* negated thermoregulation of group III proteins, and thus RacR, like some other RR proteins (27, 32, 36), responds to temperature changes. Since the physiological temperature of chickens is 42°C, the disruption of the expression of a group III protein(s) may be responsible for reduced intestinal colonization by the *racR* mutant. In addition to thermoregulation, protein 8 and Ccp (proteins 9 and 10) are under iron regulation (37), and therefore, the *racR* phenotype may be influenced by interaction with different regulatory pathways. However, as determined by 2-D gel electrophoresis (data not shown)
shown), RacR does not respond to changes in iron levels. The expression pattern of group I and II proteins indicates that some RacR regulon members are not thermoregulated. Therefore, in addition to temperature, RacR-RacS signal transduction may be influenced by other environmental conditions.

Thermoregulation in bacteria is often controlled by complex, overlapping systems that exert pleiotropic effects on the bacterial cell. Temperature-dependent regulation is important in vivo bacterial responses (10, 13), and the AraC-like family of bacterial cell signal transduction proteins (38) plays an important role in C. jejuni.

The determinants of colonization by C. jejuni are presumably expressed in response to conditions encountered in intestinal microenvironments. RacR and RacR-RacS-dependent genes are important for growth and survival in the avian intestine, and RacR-RacS is a signal transduction system responsive to temperature. Given the likely exposure of C. jejuni to temperature stress, RacR-RacS may also be required during the transmission of the bacterium from the intestine to the environmental reservoirs and vice versa. A C. jejuni strain is adjacent to racR, and we have evidence that the gene is under the transcriptional control of RacR (data not shown). C. jejuni is also required for efficient avian colonization (21), and dnaJ corresponding to the N terminus have been deposited in the GenBank database under accession no. AF053960 and AF053961, respectively.

Nucleotide sequence accession numbers. The complete nucleotide sequence of racR and the nucleotide sequence of racS corresponding to the N terminus have been deposited in the GenBank database under accession no. AF053960 and AF053961, respectively.

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Table 1. Protein profiles of the parental strain and racR mutants

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* a, expressed at a lower level.

REFERENCES


