Cloning, nucleotide sequence and characterization of a gene encoding superoxide dismutase from *Campylobacter jejuni* and *Campylobacter coli*

Desmond Purdy and Simon F. Park

Author for correspondence: Simon F. Park. Tel: +44 734 357227. Fax: +44 734 267291.

Institute of Food Research,
Reading Laboratory, Earley
Gate, Whiteknights Road,
Reading RG6 2EF, UK

Genes encoding superoxide dismutase (SOD: EC 1.15.1.1) were cloned from *Campylobacter jejuni* NCTC 11351 and *Campylobacter coli* UA585 by heterologous complementation of a SOD-deficient *Escherichia coli* mutant. Deletion analysis of the cloned *C. jejuni* DNA assigned the *sod* gene to a 1.2 kb insert and this contained an open reading frame of 660 bp. The deduced gene product of 220 amino acids was 71% identical to the *E. coli* iron-containing SOD and 60% identical to the *E. coli* manganese-containing SOD. The recombinant SOD was expressed at high levels in *E. coli* and protected a *sodA sodB* double mutant from the toxic effects of methyl viologen. Nucleotide sequence analysis of the corresponding gene from *C. coli* showed it to be 92% identical to that from *C. jejuni*.

Keywords: *Campylobacter jejuni*, *Campylobacter coli*, superoxide dismutase, cloning

INTRODUCTION

Species of the genus *Campylobacter* are some of the most prevalent pathogens causing acute gastroenteritis in man (ACMSF, 1993; Walker *et al.*, 1986). *Campylobacter*s have limited aerotolerance and are widely recognized as being microaerophilic (Hoffman, 1979a, b). A recent study (Jones *et al.*, 1993), however, has suggested the possibility that these organisms are capable of surviving for extended periods in the environment after adaptation to an aerobic metabolism. Prolonged survival of any organism in air necessarily entails mechanisms to eliminate the deleterious effects produced by exposure to superoxide radicals. In this respect, superoxide dismutase (SOD: EC 1.15.1.1), which is found in almost all organisms exposed to oxygen (Fridovich, 1986), catalyses the dismutation of superoxide radicals and is thought to form part of the defence mechanism which protects cells from oxygen toxicity (Fridovich, 1978). Accordingly, mutants of *Escherichia coli* which are deficient in SOD activity are hypersensitive to conditions of oxidative stress and consequently cannot grow aerobically when cultivated in minimal media (Carlioz & Touati, 1986). Because of its role in cellular defence against oxidative stress, SOD is

also considered to contribute to the pathogenicity of certain intracellular bacterial pathogens since macrophages can kill ingested bacteria by oxidative mechanisms (Haas & Goebel, 1992). Accordingly, mutants of *Shigella flexneri* which are deficient in a manganese-containing SOD show decreased survival in macrophages (Franzon *et al.*, 1990).

Studies with other bacterial species have highlighted the important role SOD plays in the physiology and pathogenicity of bacterial pathogens (Haas & Goebel, 1992). To date, however, no attempts have been made to characterize the genes encoding SOD in any *Campylobacter* spp. or indeed any other microaerophilic bacterium. Previously, SOD activity has been detected in 23 strains of *Campylobacter* spp. (Kikuchi & Suzuki, 1984) and it has been suggested that *C. jejuni* may possess an iron-requiring SOD (Hoffman *et al.*, 1979a). To assess the contribution of SOD to the survival of campylobacters in air and during infection we have cloned the genes encoding SOD from *Campylobacter jejuni* and *C. coli*, the most frequently isolated campylobacters associated with diarrhoeal disease (Griffiths & Park, 1990). We describe the nucleotide sequence of the genes and their expression in *E. coli* as enzymically active recombinant proteins.

METHODS

Bacterial strains and plasmids. *C. jejuni* NCTC 11351 (Type strain) was purchased from the National Collection of Type

Abbreviation: SOD, superoxide dismutase.

The GenBank accession numbers for the sequences reported in this paper are X76692 (*Campylobacter jejuni*) and X76693 (*C. coli*).

Cultures (Colindale) and *C. coli* UA585 was a generous gift from D. Taylor (University of Alberta, Edmonton). *Escherichia coli* QC1799, ($F^- \Delta lac4169 rpsL \Delta sodA3 \Delta sodB2$ Km^r) a *sodA sodB* double mutant incapable of growth on minimal medium containing methyl viologen, was kindly supplied by D. Touati (Institute Jacques Monod, Paris). The plasmid pTZ19R (Mead *et al.*, 1986), used as a general cloning vector, was supplied by Pharmacia. *Campylobacters* were grown at 37 °C on Mueller–Hinton (MH) agar, under microaerophilic conditions using the recommended gas generating kit (Oxoid). *E. coli* QC1799 was routinely cultured on LB agar plates (Maniatis *et al.*, 1982). When necessary, ampicillin was included in growth media at 100 µg ml⁻¹ to maintain plasmid selection.

Cloning and DNA analysis. Genomic DNA was isolated from campylobacters using guanidium thiocyanate (Pitcher *et al.*, 1989). Plasmid libraries of chromosomal DNA from *C. jejuni* and *C. coli* were established in dephosphorylated pTZ19R using genomic DNA partially digested with *Hind*III, as described by Maniatis *et al.* (1982). Following ligation, libraries were used to transform *E. coli* QC1799 by electroporation as described by Dower *et al.* (1988). After transformation, cells were washed three times in sterile $\frac{1}{4}$ strength Ringers solution before plating. Transformants were selected by aerobic growth on M9 minimal agar (Maniatis *et al.*, 1982) containing methyl viologen (0.05 µM) and ampicillin (100 µg ml⁻¹) as described by Carlouz & Touati (1986).

Plasmid deletion was carried out using an exonuclease III/mung bean nuclease kit following digestion of pJSOD1 with *Kpn*I and *Xba*I (Northumbria Biologicals). A series of nested plasmid deletions, prepared in this manner, was then used to transform *E. coli* QC1799 to determine the smallest size of DNA insert which conveyed resistance to methyl viologen using the selection procedure described above.

Nucleotide sequence analysis was carried out by the dideoxy chain termination method (Sanger *et al.*, 1977), using a Sequenase version 2.0 kit (USB). Initially, nucleotide sequence was determined from both DNA strands using the M13 universal forward and reverse primer sites and the deletion plasmid pDEL1 as template. Further oligonucleotide primers of approximately 20 nucleotides were designed according to the sequence information and synthesized using a 391 DNA synthesizer (Applied Biosystems). The sequence of the *C. coli sod* gene was determined using these same primers. Computer analysis of the sequence data was carried out using the Wisconsin molecular biology software package (Devereux *et al.*, 1984).

DNA/DNA hybridizations. DNA/DNA hybridizations were carried out using a non-radioactive ECL gene detection kit (Amersham). Chromosomal DNA was restricted using either *Hind*III or *Pst*I and electrophoresed on a 0.8% agarose gel. After depurination of the DNA, using 250 mM HCl, DNA was transferred to Hybond N+ nitrocellulose membranes (Amersham) using an alkali transfer procedure (Maniatis *et al.*, 1982). A DNA probe, generated using a 666 bp *Hind*III fragment from pDEL1, was then used to detect homology.

Protein extraction and analysis. Crude cell lysates of *E. coli* in L-broth or *C. jejuni* grown microaerophilically on MH agar were prepared from overnight cultures. Harvested cells were washed twice in 0.15 M Tris/HCl, pH 7.0, and resuspended in 0.15 M Tris/HCl, pH 7.0, containing 0.2 mg lysozyme ml⁻¹ and 0.1 mM EDTA. Five cycles of freeze thawing, using a com-

bination of liquid N₂ and 42 °C, were used to lyse the cells. The lysates were then cleared by centrifugation at 20000 g for 20 min. Soluble cellular proteins were extracted in the supernatant.

Cell lysates were electrophoresed under both denaturing and non-denaturing conditions (Laemmli, 1970). Under non-denaturing conditions samples were not boiled and SDS and β-mercaptoethanol were omitted from all buffers. Gels were stained for SOD activity using the method described by Beauchamp & Fridovich (1971).

RESULTS

Cloning of the *sod* gene from *C. jejuni* NCTC 11351

The *E. coli* strain QC1799 is a *sodA sodB* double mutant and is, consequently, unable to grow aerobically when cultivated on minimal medium supplemented with methyl viologen (Carlouz & Touati, 1986). A genomic library of DNA from *C. jejuni* NCTC 11351, which consisted of approximately 10000 independent clones with inserts of approximately 6.0 kb, was constructed in *E. coli* QC1799. One transformant capable of growth on minimal media containing methyl viologen was isolated. This clone was found to harbour a plasmid, which contained an insert of approximately 4.5 kb, and was designated pJSOD1.

Localization of the *sod* gene in pJSOD1

To locate the gene encoding the SOD activity, and to simplify the ensuing nucleotide sequence determination, unidirectional deletions were generated in pJSOD1 using exonuclease III and mung bean nuclease. The deletion derivatives generated were then used to transform *E. coli* QC1799 and selection made for the ability to complement the SOD-deficient phenotype of this strain. The smallest plasmid capable of conferring resistance to the toxic effects of methyl viologen retained a 1.2 kb insert of *C. jejuni* DNA (data not shown). This plasmid, designated pDEL1, was used as template for nucleotide sequence determination.

Nucleotide sequence of the *sod* gene from *C. jejuni*

Nucleotide sequence analysis of the DNA insert in pDEL1 revealed an open reading frame of 660 bp which could encode a potential polypeptide of 220 amino acids (Fig. 1) with a calculated molecular mass of 24804 Da. This corresponds well with the molecular mass of a protein of approximately 23.5 kDa which was detected by denaturing SDS-PAGE in extracts of cells containing pDEL1 and pJSOD1 (see later). The nucleotide sequence of the putative *C. jejuni sod* gene shows 57% identity to the *E. coli* iron-containing SOD and 45% identity to the manganese-containing SOD. A putative ribosome binding site exists 11 bp upstream of the ATG start codon (Gold, 1988). Putative -10 and -35 sites may also exist 49 bp and 71 bp upstream of the ATG start codon, respectively. These show homology to the consensus sequences predicted by Hawley & McClure (1983). The G + C content of the coding sequence is 32.6%, which is

AAAAAATATAATTTTTTATATATTTAAATATTATTTAAATATATTTAAATGTATC 60
 TTATTTTATTTAGGTTAAATAAATAAGAAATTTTACCACCTAAGCTTTTAAAGATTTTTTA 120
 CTCCTTTTAAATATTATTATGGTAATATTTTCAATATCAAACTAAAAATAAGGAGAAATT 180
 ATGTTTGAATTAAGAAAAATACCTTATGATACCAATGCTTTTGGTGATTTTTTGAAGTCT 240
 M F E L R K L P Y D T N A F G D F L S A
 GAACTTTTAGCTATCATCATGGAACATCAACAATCTTATGTTACAAATCTAAATAAT 300
 A T F S Y H H G K H H N T Y V T N L N N
 CTTATTAAGATCTGAATTTGAGGTAAGATCTTGTAGTATTATCAAACTTCAAAAT 360
 L I K D T E F A G K D L V S I I K T S N
 GGGGCGCTATTTAAGCAGCTCAAGTTTATAATCATGATTTTTTATTTGATTGCATT 420
 G G V F N N A A Q V Y N H D F Y F D C I
 AAGCAAAGTACAGCTGTGGCTGAGGTTTGTAGTCAAGTATAGATGCTAATTTACAA 480
 K P S T G C G C G G S C Q S I D A N L Q
 GCGGCATAGAAAAAGAAATTTGGATCTTTAGAAAATTTCAAAGCTGAATTCATAAGGT 540
 A A L E K E F G S L E N F K A E F I K G
 GCTACAGGAGTTTTTGGTTTCAGGATGTTTGGTTAGTTTATAACTAAAAATCAAAA 600
 A T G V F G S G W F W L V Y N T K N Q K
 CTAGAATTTGTAGTACTTCAACGACGCTACACCAAACTAGAGATAAAGTCTCTTA 660
 L E F V G T S N A A T P N T E D K V P L
 CTTGTTGATGTTTGGGAACATGCTTATTATGATCATCGTAATGCACGCCCTGCT 720
 L V V D V W E H A Y Y V D H R N A R P A
 TATTAGAAAAATCTATGCTCATATTAAGTGGGAATTTGTTGCAAAAGCTTACGAATGG 780
 Y L E K F Y A H I N W E F V A K A Y E W
 GCTTTAAAAAGGATGGGATGAGTCTTATGCAAAATGAACCTTCACTCTGTAATA 840
 L K E G M G S V S F Y A N E L H S V K
 TAACCTTAATCTTTTGTGCTAGATGGATTTGACATTAGTCAACATCTACACAAA 900

Fig. 1. Nucleotide sequence and amino acid translation of the *sod* gene of *C. jejuni* NCTC 11351. The nucleotide sequence of a 900 bp region of the 1.2 kb insert in pDEL1 is shown. A potential RBS is underlined. Promoter sequences homologous to the -10 and -35 regions of *E. coli* are denoted by horizontal lines above the sequence. Stop codons are shown by asterisks.

consistent with the low G + C content of all *Campylobacter* species (Belland & Trust, 1982).

Comparison of the putative amino acid sequence of the *C. jejuni* *sod* gene product with previously characterized sequences revealed 71 % and 60 % identity with the *E. coli* iron-dependent SOD and manganese-dependent SOD (Fig. 2) respectively. Furthermore, comparison of the putative amino acid sequence with the iron and manganese classes of SOD, as described by Parker & Blake (1988), points to the cloned SOD having iron as its metal cofactor. The amino acid sequences of the cloned SOD gene matches the expected amino acid residue at four of the five primary determinants and 13 of the 17 secondary determinant residues predicted for iron containing SODs (Table 1).

Sequence analysis of the *C. coli* *sod* gene

To compare the gene encoding SOD from *C. jejuni* with that from another species of *Campylobacter*, the *sod* gene from *C. coli* UA585 was cloned in an analogous manner (data not shown). Nucleotide sequence analysis revealed a 660 bp open reading frame which could encode a polypeptide 220 amino acids long (Fig. 2). The nucleotide sequence of the open reading frame showed 92 % identity

Human (Mn)	KHSLPDLFYPDYGALEPHINAQIMQLHSHKHAAYVNNLVTEEKY
<i>E. coli</i> (Mn)	SYTLPSLPYAYDALEPHFDKQTEIHHTKHQTYVNNNAALSL
<i>E. coli</i> (Fe)	SEFLPALPYAKDALAPHISAETIEYHYGKHQTYVNNLNLIKGT
<i>C. jejuni</i>	-FELRKLFPYDTNAGDFLSAETFSYHHGKHHTTYVNNLNLIKDT
<i>C. coli</i>	-FELRKLFPYDTNAGDFLSAETFSYHHGKHHTTYVNNLNLIKYT
Human (Mn)	QEALAKGDVTAQI-----ALQPAKFNGGGHINHSIFWNLSP-
<i>E. coli</i> (Mn)	PEFANLPVEELITKLQPADKKTVLRRNAGGHAHNSLFWKGLKK-
<i>E. coli</i> (Fe)	-AFEGKSLLEEII-R-----SSEGGVFNNAAQVNNHTFYVNCCLAP-
<i>C. jejuni</i>	-EFAGKDLVSII-K-----TSNGGVFNNAQVYNNHDFYFDCIKP-
<i>C. coli</i>	-EFASKDLVSII-K-----SSGGVFNNAAQVYNNHDFYFDCIKP-
Human (Mn)	-----NGGGE-P-K-GELLEAIKRDFFSGDFKFEKLTAAASVGVS
<i>E. coli</i> (Mn)	-----GTTL-Q-----GDLKPAIERDFGSDVDFNKAEFKAAASRFGSG
<i>E. coli</i> (Fe)	-----NAGGE-P-T-GKVAEAIASFGSADFKAQFTDAAIKNFGSG
<i>C. jejuni</i>	ITGCGCGGSCQSIDANLQAALKEFGSLENFKAEIFKATGVFVFGSG
<i>C. coli</i>	ITGCGCGGSCQSM DANLQAALKEFGSLENFKAEIFKATGVFVFGSG
Human (Mn)	WGWLGFN-KERGHQLQIAACPNDQ-PLQGT-----GLIPLLGLDVWE
<i>E. coli</i> (Mn)	WAWLVLK---GDKLAAVSTANDQSLMGEAISGASGSPILWLGWVE
<i>E. coli</i> (Fe)	WTWLKVN-S-DGKLAIYSTSNAGTPLTTDA-----TPLLTVDFWE
<i>C. jejuni</i>	WFVLVYNTKNQ-KLEFVGTSNAATPNTED-----KVPLLVDVWE
<i>C. coli</i>	WFVLVYNTKNQ-KLEFVGTSNAATPNTED-----KVPLLVDVWE
Human (Mn)	HAYYLQYKYNVRPDLKAIWNVINWENVTERY-MACK-K
<i>E. coli</i> (Mn)	HAYYLKFNRRPDYIKFVNVVNWDEAAARF-AAAK-K
<i>E. coli</i> (Fe)	HAYYIDYRNARPGYLEHFWLVNWFVAKNL-AA----
<i>C. jejuni</i>	HAYYVDHRNARPAYLEKFAHINWEEFVAKAYEWALK--EGMGSVS
<i>C. coli</i>	HAYYVDHRNARPAYLEKFAHINWEEFVAKAYEWALK--EGMGSVS
<i>C. jejuni</i>	FYANELHSVK
<i>C. coli</i>	FYANELHPVK

Fig. 2. Comparison of the SOD from *C. jejuni* and *C. coli* with SOD from other organisms. The derived amino acid sequences from *C. jejuni* and *C. coli* are aligned with human MnSOD and both Mn- and Fe-type SODs from *E. coli*. Residues conserved between all five enzymes are shown in bold.

with that from *C. jejuni* (Fig. 3), whilst the putative amino acid sequence of the gene product showed 97 % identity with that of *C. jejuni* (Fig. 2). In addition, the amino acid residues used to classify the *C. jejuni* enzyme as an FeSOD (Parker & Blake, 1988) were entirely conserved in the *C. coli* gene product (data not shown).

Expression and electrophoretic analysis of the cloned *C. jejuni* *sod*

The orientation of the cloned open reading frame which encodes the SOD from *C. jejuni* in the vector would allow its transcription to be directed by the *lacZ* promoter of pTZ19. However, high levels of *sod* expression were observed in the absence of IPTG (data not shown) and it is possible that transcription of SOD is dependent on the recognition of a *Campylobacter* sequence as a promoter by the *E. coli* host. We cannot, however, exclude the possibility that expression of *sod* is occurring from the *lac* promoter following titration of the repressor molecule. When cell lysates from *E. coli* QC1799 harbouring pJSOD1 or pDEL1 were analysed by SDS-PAGE electrophoresis, a major band at 23.5 kDa was seen in both, but not in lysates from cells which had contained the plasmid, pTZ19R (Fig. 4). This corresponds well to the expected molecular mass of SOD (24.8 kDa) and suggests that the integrity of the open reading frame for SOD was retained in the deletion derivative pDEL1. When the equivalent cell lysates were analysed by non-denaturing PAGE and stained specifically, it was apparent that presence of SOD activity in the *sodA sodB* mutant of *E. coli* was dependent on the presence of pJSOD1 and

Table 1. Determinant residues in the *C. jejuni* SOD used to distinguish between the Fe-type and Mn-type enzyme

The amino acid residues used to distinguish between FeSOD and MnSOD are aligned and numbered according to Parker & Blake (1988). Alp, aliphatic side chain; Bas, basic side chain; Mix, more than one residue type; Aro, aromatic side chain.

Residue no.	MnSOD	FeSOD	<i>C. jejuni</i>	Match
Primary candidates				
76	Gly	Ala	Ala	Fe
77	Gly	Gln	Gln	Fe
84	Phe	Tyr	Tyr	Fe
154	Gln	Ala	Ala	Fe
155	Asp	Gly	Ala	?
Secondary candidates				
23	Met	Alp	Phe	?
25	Alp	Tyr	Tyr	Fe
45	Aro	Thr	Thr	Fe
67	Alp	Ser	Ser	Fe
69	Mix	Gly	Gly	Fe
70	Alp	Gly	Gly	Fe
72	Bas	Phe	Phe	Fe
78	His	Val	Val	Fe
79	Alp	Trp	Tyr	?
83	Alp	Phe	Phe	Fe
87	Mix	Cys	Cys	Fe
159	Mix	Thr	Thr	Fe
173	Gly	Thr	Val	?
184	Mix	Asp	Asp	Fe
186	Mix	Arg	Arg	Fe
199	Val	Leu	His	?
204	Mix	Phe	Phe	Fe

pDEL1, containing the cloned *C. jejuni* SOD (Fig. 5). Four electromorphic forms of the *sod* gene product, probably corresponding to multimeric forms of the protein (with R_F values of 0.57, 0.60, 0.63 and 0.67) were present in both *C. jejuni* and *E. coli* extracts. However, two additional bands of SOD activity (with R_F values of 0.32 and 0.44) were present in the recombinant strains of *E. coli* which may correspond to higher multimers of the protein. Similar results were obtained when cell lysates from *C. coli* UA585 and *E. coli* harbouring the cloned *sod* from the former were analysed by electrophoresis under non-denaturing conditions (data not shown).

DNA/DNA hybridizations

To confirm the presence of the *sod* gene in the genome of *C. coli* and *C. jejuni*, and to determine its copy number, a portion of the cloned *C. jejuni* *sod* gene (a 0.67 kb *Hind*III fragment) was labelled and hybridized to digested chromosomal DNA from *Campylobacter* (Fig. 6). This probe hybridized to a single *Hind*III fragment of 0.67 kb in both species and to 11.5 kb and 9.3 kb *Pst*I fragments in *C. jejuni* and *C. coli*, respectively. Since the probe

```

CTTTTATATAATTTTATGCTACAATATTTTGTCAAACCTAAATTAAGGAGAAATT 60
ATGTTTGAATTAAGAAATACCTTATGATACCAATGCTTTTGGTGATTTTTAAGTGCT 120
.....G.....
GAACTTTTAGTTATCATCATGGAAAACACCACAATCTATGTAACAAATCTTAATAAT 180
.....C.....T.....T.....A.....
CTTATCAATATACTGAATTGCAAGTAAAGATCTTGAAGCATTATTAAGTTCAAGT 240
.....T...G.....G.....T.....C.....C.....A.....
GGTGGAGTATTTAACAATGCTGCTCAAGTTTATAATCATGATTTTATTTGATTGCATT 300
..G..C.....T..C..A.....
AAACCGATCAGAGCTGTGGCTGTGGTGCATGCCAAAGTGGATGCTAATTACAA 360
..G..A..GT.....C..T.....T.....A.....A.....
GCAGCTTTAGAAAAAGAAATTTGGTCTTTAGAGAAATTCAAAGCTGAATTTATCAAGGT 420
..G..AC.....A.....A.....A.....C.....
GCGACAGGGGTTTTTGGTTCAGGTGGTTTGGTTAGTTTATAACTATAAAATCAAAA 480
..T....A.....A.....
TTAGAATTTGTGGTACTTCAATGCAAGTACACCTATCACTGAAGATAAAGTTCCITTA 540
C.....A.....C.....A..AT.....
CTTGTGTGGATGTTTGGGAGCATGCTTATGTTAGATCATGTAATGCCGCTCCTGGC 600
.....A.....A.....A.....A.....C.....T
TATTAGAAAAATCTATGCTCATATTAACTGGGAATTTAGCAAAAGCTTATGAATGG 660
.....T.....C.....
GCTTTAAAGAAGGTATGGGATCAGTTAGCTTTTACGCAACGAACTTCATCCTGTAATA 720
.....C.....T.....T.....CT.....
TAATACAAGGCATCTTAAAGATGCCTTTTACTCTCCTAACTTGGAGTCATTTCGATA 780
*                               *

```

Fig. 3. Complete nucleotide sequence of the *sod* gene of *C. coli* UA585. The ATG start for the open reading frame is denoted by underlining and the derived amino acid sequence translated from this point is shown in Fig. 2. Stop codons are shown as asterisks. The sequence of the same region of DNA from *C. jejuni* NCTC 11351 is aligned beneath. Only the differences between the *C. coli* and *C. jejuni* sequences are shown.

Fig. 4. Expression of the *sod* gene from *C. jejuni* in *E. coli*. Total soluble proteins from recombinant strains of *E. coli* QC1799 were analysed by electrophoresis on a 10% SDS-PAGE and visualized by Coomassie blue staining. Lanes: 1, molecular mass markers; 2, lysate from *E. coli* QC1799; 3, lysates from *E. coli* QC1799 containing plasmids pTZ19; 4, pJSOD1; and 5, pDEL1.

hybridized to a single band in both digests of *Campylobacter* DNA it is likely that both campylobacter species possess only a single copy of this *sod* gene.

Fig. 5. Detection of SOD activity in *C. jejuni* and *E. coli* QC1799 containing recombinant plasmids. Total soluble proteins from *C. jejuni* and recombinant strains of *E. coli* QC1799 were analysed by electrophoresis on a 7.5% non-denaturing polyacrylamide gel and visualized following specific staining for SOD activity (Beauchamp & Fridovich, 1971). The positions of *E. coli* MnSOD and FeSOD are shown in lanes 1 and 2. Lane 3 shows a cell lysate from *C. jejuni*. Cell lysates from *E. coli* QC1799 containing the plasmids pJSOD1 (lane 4), pDEL1 (lane 5) and pTZ19R (lane 6) are also shown.

Fig. 6. Hybridization of part of the *C. jejuni* *sod* gene to chromosomal DNA from *C. jejuni* and *C. coli*. A 0.67 kb *Hind*III fragment was labelled and used to probe genomic DNA from *C. jejuni* digested with *Hind*III (lane 1) and *Pst*I (lane 3). Genomic DNA from *C. coli* digested with *Hind*III (lane 2) and *Pst*I (lane 4) was also used as a target.

DISCUSSION

SOD is common to most aerobic organisms and has been implicated in the survival of bacteria under aerobic conditions (Fridovich, 1986; Carliz & Touati, 1986). In this study we have cloned the gene for a putative FeSOD from both *C. jejuni* and *C. coli* by complementation of a SOD-deficient mutant of *E. coli*. Despite the often encountered problems in cloning DNA from *Campylobacter* into *E. coli* (Taylor, 1992) the genes are expressed efficiently and appear to be maintained stably in *E. coli*. Characterization of the nucleotide sequence encoding the SOD activity from *C. jejuni* revealed an open reading frame of 660 bp which could encode a putative gene product of 220 amino acids and which showed 71% and 60% identity with the *E. coli* FeSOD and MnSOD, respectively. Sequence analysis of a wide range of MnSODs and FeSODs has identified a number of key residues which are characteristic of the enzymes which utilize different metal ligands. Accordingly, analysis of aligned amino acid sequences may be used to distinguish between Fe- and MnSODs on the basis of a number of primary and secondary single-site substitutions (Parker & Blake, 1988). The amino acid sequences of the putative

SODs cloned from both *C. jejuni* and *C. coli* indicate that 17 out of 22 determinant residues are the same as for FeSODs. It is likely, therefore, that iron is the metal co-factor for both enzymes. Moreover, since no obvious band of MnSOD activity was observed when cell lysates were examined by non-denaturing electrophoresis it is possible that the iron-type enzyme is the only SOD possessed by these strains of *Campylobacter*. However, we cannot exclude the possibility that these strains contain an inducible MnSOD which is not expressed under the microaerophilic conditions used to culture the cells. The FeSOD of *C. jejuni* was present in four electromorphic forms when analysed under non-denaturing conditions. Equivalent forms of SOD were also present in lysates of *E. coli* containing the recombinant SOD. The simplest explanation for these bands of different mobility is that they represent multimeric forms of the protein containing one, two, three and four subunits. This is consistent with observations made with the SOD from other thermophilic bacteria (Fridovich, 1986) and mycobacterial species (Zhang *et al.*, 1991) which form trimers and tetramers.

Given the microaerophilic nature of *Campylobacter* spp., the survival of these organisms under conditions of

oxidative stress must be profoundly influenced by the presence of cellular mechanisms which can eliminate reactive oxygen intermediates. As SOD is part of the defence mechanism which protects cells from oxidative stress (Fridovich, 1986) it is likely to play a crucial role in the survival of campylobacters in aerobic environments in which reactive oxygen intermediates are generated. For example, the inclusion of bovine SOD in the growth medium greatly enhances the oxygen tolerance of *C. jejuni* (Hoffman, 1979b) and it has been suggested that the aerotolerance in *Campylobacter* spp. increases with elevated SOD activity (Kikuchi & Suzuki, 1984). Having characterized the genes encoding this enzyme we are now in a position to generate isogenic mutants deficient in SOD by gene replacement (Labigne-Roussel *et al.*, 1988) so that the role of SOD in the physiology and virulence of this group of clinically important pathogens can be investigated.

ACKNOWLEDGEMENTS

We are grateful to D. Touati and D. E. Taylor for the generous gifts of bacterial strains. We thank the Ministry of Agriculture, Fisheries and Foods for financial support for this work.

REFERENCES

- ACMSF (1993).** Advisory Committee on the Microbiological Safety of Foods, Interim report on *Campylobacter*. HMSO.
- Beauchamp, C. & Fridovich, I. (1971).** Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Anal Biochem* **44**, 276–287.
- Belland, R. J. & Trust, T. J. (1982).** Deoxyribonucleic acid sequence relatedness between thermophilic members of the genus *Campylobacter*. *J Gen Microbiol* **128**, 2515–2522.
- Carlioz, A. & Touati, D. (1986).** Isolation of superoxide dismutase mutants in *Escherichia coli*: is superoxide necessary for aerobic life? *EMBO J* **5**, 623–630.
- Deveureux, J., Haeberli, P. & Smithies, P. (1984).** A comprehensive set of sequence analysis programs for the VAX. *Nucleic Acids Res* **12**, 387–395.
- Dower, W. J., Miller, J. F. & Ragsdale, C. W. (1988).** High efficiency transformation of *E. coli* by high voltage electroporation. *Nucleic Acids Res* **16**, 6127–6145.
- Franzon, V. L., Arondel, J. & Sansonetti, P. J. (1990).** Contribution of superoxide dismutase and catalase activities to *Shigella flexneri* pathogenesis. *Infect Immun* **58**, 529–535.
- Fridovich, I. (1978).** The biology of oxygen radicals. *Science* **201**, 875–880.
- Fridovich, I. (1986).** Superoxide dismutases. *Adv Enzymol* **58**, 61–97.
- Gold, L. (1988).** Post-transcriptional regulatory mechanisms in *Escherichia coli*. *Annu Rev Biochem* **57**, 199–233.
- Griffiths, P. L. & Park, R. W. A. (1990).** Campylobacters associated with human diarrhoeal disease. *J Appl Bacteriol* **69**, 281–301.
- Haas, A. & Goebel, W. (1992).** Microbial strategies to prevent oxygen dependent killing. *Free Radical Res Commun* **16**, 137–157.
- Hawley, D. K. & McClure, W. R. (1983).** Compilation and analysis of *Escherichia coli* DNA promoter sequences. *Nucleic Acids Res* **11**, 2237–2255.
- Hoffman, P. S., George, H. A., Kreig, N. R. & Smibert, R. M. (1979a).** Studies of the microaerophilic nature of *Campylobacter fetus* subsp. *jejuni*. I. Physiological aspects of enhanced aerotolerance. *Can J Microbiol* **25**, 1–7.
- Hoffman, P. S., George, H. A., Kreig, N. R. & Smibert, R. M. (1979b).** Studies of the microaerophilic nature of *Campylobacter fetus* subsp. *jejuni*. II. Role of exogenous superoxide anions and hydrogen peroxide. *Can J Microbiol* **25**, 8–16.
- Jones, D. M., Sutcliffe, E. M., Rios, R., Fox, A. J. & Curry, A. (1993).** *Campylobacter jejuni* adapts to aerobic metabolism in the environment. *J Med Microbiol* **38**, 145–150.
- Kikuchi, H. E. & Suzuki, T. (1984).** An electrophoretic analysis of superoxide dismutase in *Campylobacter* spp. *J Gen Microbiol* **130**, 2791–2796.
- Labigne-Roussel, A., Courcoux, P. & Tompkins, L. (1988).** Gene disruption and replacement as a feasible approach for mutagenesis of *Campylobacter jejuni*. *J Bacteriol* **170**, 1704–1708.
- Laemmli, U. K. (1970).** Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680–685.
- Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982).** *Molecular Cloning. A Laboratory Manual*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Mead, D. A., Szczena-Skorupa, E. & Kemper, B. (1986).** Single-stranded 'blue' T7 promoter plasmids: a versatile tandem promoter system for cloning and protein engineering. *Protein Engineering* **1**, 67–74.
- Parker, M. W. & Blake, C. C. F. (1988).** Iron- and manganese-containing superoxide dismutases can be distinguished by analysis of their primary structures. *FEBS Lett* **229**, 377–382.
- Pitcher, D. G., Saunders, N. A. & Owen, R. J. (1989).** Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. *Lett Appl Microbiol* **8**, 151–156.
- Sanger, F., Nicklen, S. & Coulson, A. R. (1977).** DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci USA* **74**, 5463–5467.
- Taylor, D. E. (1992).** Genetics of *Campylobacter* and *Helicobacter*. *Annu Rev Microbiol* **46**, 35–64.
- Walker, R. I., Caldwell, M. B., Lee, E. C., Guerry, P., Trust, T. J. & Ruiz-Palacios, G. M. (1986).** Pathophysiology of *Campylobacter* enteritis. *Microbiol Rev* **50**, 81–94.
- Zhang, Y., Lathigra, R., Garbe, T., Catty, D. & Young, D. (1991).** Genetic analysis of superoxide dismutase, the 23 kilodalton antigen of *Mycobacterium tuberculosis*. *Mol Microbiol* **5**, 381–391.

Received 19 October 1993; revised 30 November 1993; accepted 6 December 1993.