Occurrence of two superoxide dismutases in *Aeromonas hydrophila*: molecular cloning and differential expression of the *sodA* and *sodB* genes

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***Aeromonas* spp., considered as emerging opportunistic pathogens, belong to the family *Vibrionaceae*. Among the criteria currently used for their classification is the presence of a single FeSOD (iron-containing superoxide dismutase), which distinguishes them from *Enterobacteriaceae*. In this paper the cloning of the *sodA* and *sodB* genes encoding two different SODs in *Aeromonas hydrophila* ATCC 7966 is reported. The *sodB* gene encoded an FeSOD (196 amino acids, 21.5 kDa), was constitutively expressed and showed 75% homology with the *E. coli* FeSOD. The *sodA* gene encoded a protein of 206 amino acids (22.5 kDa) with MnSOD (manganese-containing SOD) activity and showed 55% homology with the *Escherichia coli* MnSOD. The MnSOD of *A. hydrophila* was detected only during the stationary phase of growth under high aeration or when induced by lack of iron. Nevertheless, paraquat had no detectable effect on its production. The amino-terminal part of the Mn-containing protein contained a putative signal sequence which could permit a periplasmic localization.**

Keywords: *Vibrionaceae*, MnSOD, FeSOD

INTRODUCTION

Antioxidant enzymes are essential for living cells, providing protection from reactive oxygen species such as superoxide, which cause oxidative damage to cell structures. Superoxide dismutases (SODs; EC 1.15.1.1) constitute a ubiquitous class of antioxidant defence metalloenzymes that catalyse the conversion of superoxide radical ion into dioxygen and hydrogen peroxide.

The SODs are classified according to the metal ion cofactor required for their activity: the copper-zinc type (Cu/ZnSOD), the manganese type (MnSOD), the iron type (FeSOD) (Fridovich, 1986) and the most recently described nickel type (NiSOD) (Youn *et al.*, 1996). Bacteria contain one to three SOD enzymes, which can

be expressed simultaneously. The facultative anaerobe *Escherichia coli* possesses three SODs which differ in their location and temporal expression. Both FeSOD and MnSOD are cytoplasmic. FeSOD is produced at a constant rate under aerobic and anaerobic conditions, but MnSOD is only synthesized aerobically and its presence is modulated by exposure to oxygen or intracellular O₂⁻ or upon changes in growth phase (Demple, 1991; Compan & Touati, 1993). The third *E. coli* SOD, containing Cu/Zn, is located within the periplasmic space (Benov *et al.*, 1995).

Bacteria belonging to the genus *Aeromonas* are Gram-negative facultatively anaerobic rods displaying catalase and oxidase activities. They are currently classified in the family *Vibrionaceae*, but a separate family has been proposed on the basis of the 16S rRNA cataloguing and rRNA–DNA hybridization results (Colwell *et al.*, 1986). They are commonly found in aquatic environments and increasingly in ready-to-eat foods (Kirov, 1997). They are considered as emerging opportunistic pathogens associated with gastroenteritis and enterocolitis in humans (Merino *et al.*, 1995).

Abbreviations: CDM, chemically defined medium; DIP, 2,2'-dipyridyl; EDDA, ethylenediamine di-(o-hydroxyphenylacetic acid); PQ, paraquat; SOD, superoxide dismutase.

The GenBank accession numbers for the sequences reported in this paper are AF317226 and AF317227.

According to the description of the family *Vibrionaceae* in *Bergey's Manual of Systematic Bacteriology* (Baumann & Schubert, 1984), *Aeromonas* expresses a single FeSOD and this feature tends to discriminate between the families *Vibrionaceae* and *Enterobacteriaceae*.

In this study we identified and characterized the genes encoding SODs from *Aeromonas hydrophila* and compared their expression to that of the *E. coli* SODs.

METHODS

Strains, media and culture conditions. The strains and plasmids used in this study are listed in Table 1. *E. coli* and *A. hydrophila* were grown in LB medium (per litre: tryptone 10 g; yeast extract 5 g; NaCl 5 g; pH 7.2) at 30 °C. For iron-limited cultures, the glassware was treated with 10% (v/v) nitric acid, and the cells were grown either (i) in LB medium containing iron chelators (each at 100 µM): DIP (2,2'-dipyridyl), EDDA [ethylenediamine di-(o-hydroxyphenylacetic acid)], desferrioxamine B (Desferal; Ciba-Geigy) or (ii) in CDM (per litre: glucose 5 g; (NH₄)₂HPO₄ 1 g; K₂HPO₄ 4 g; KH₂PO₄ 2.7 g) made with Chelex-100-treated distilled water and supplemented after sterilization with sulfate salts of magnesium (830 µM) and manganese (40 µM). Broth cultures were grown under high aeration (100 ml medium in a 1 litre flask with vigorous rotary shaking at 100 r.p.m.) or under low aeration (100 ml medium in a 300 ml flask with gentle swirling at 40 r.p.m.).

Cloning and sequencing of *sod* genes. Chromosomal DNA from *A. hydrophila* was prepared from an overnight culture in 5 ml LB medium. DNA was partially digested with *Sau*3A and fragments ranging from 2 to 8 kb were extracted from a 0.8% agarose gel using the Qiaquick Gel Extraction kit. These fragments were ligated into the dephosphorylated *Bam*HI site of pUC19. The recombinant plasmids were transformed into the *E. coli* SOD⁻ mutant QC871 and plated onto LB agar containing ampicillin (50 µg ml⁻¹) and 10 µM paraquat (PQ). Chromosomal and plasmid DNA were prepared using the corresponding Qiagen kits. The inserts were sequenced by the ABI PRISM BigDye terminator cycle sequencing system (Perkin-Elmer).

Preparation of bacterial extracts and assays. The cells were harvested by centrifugation at 12000 g for 15 min at 4 °C, washed with and suspended in 50 mM sodium phosphate buffer pH 7.8, then stored at -20 °C. Cells were disrupted by sonication, and after centrifugation at 12000 g for 15 min at 4 °C, the supernatants (crude extracts) were stored at 4 °C for immediate use or frozen at -20 °C.

Protein concentration was determined using a Bio-Rad DC protein assay kit. Total SOD activity from mid-exponential-phase crude extracts was estimated using the xanthine/xanthine oxidase procedure (Beauchamp & Fridovich, 1971). The amount of SOD required to inhibit the reduction rate of nitro blue tetrazolium by 50% was defined as one unit of activity.

The number and nature of SODs were determined by the PAGE method already described (Leclère *et al.*, 1999). Triplicate gels were soaked in riboflavine containing 5 mM H₂O₂ or 2 mM KCN to differentiate between Fe- and MnSOD (Droillard *et al.*, 1989).

Cell fractionation by osmotic shock. Bacterial cells harvested after 30 h growth in LB medium were washed twice with 10 mM Tris. The cells were suspended in 10 mM Tris/20% sucrose and stirred at room temperature for 15 min, then suspended in the same buffer without sucrose and stirred again for 15 min in ice. The periplasmic fraction was obtained by centrifugation at 15000 g for 30 min at 4 °C and the cells were disrupted by sonication to get the cytosolic fraction.

RESULTS

Gene cloning and expression of SODs

The molecular cloning of the *sod* genes from *A. hydrophila* ATCC 7966 was achieved by genetic complementation of the *E. coli* QC871 *sodA sodB* double mutant. Two clones named EcA125 and EcA126 were selected; the corresponding plasmids pVLAh125 and pVLAh126 contained a 2.9 kb and 3.6 kb insert, respectively.

The ability of the plasmids to restore resistance to PQ to the SOD⁻ double mutant of *E. coli* was studied by

Table 1. Bacterial strains and plasmids

Strain or plasmid	Genotype and/or description	Source or ref.
Plasmids		
pUC19	Cloning vector; Ap ^R	Lab. stock
pVLAh125	pUC19 with 2.9 kb insert from <i>A. hydrophila</i> 7966; expresses an FeSOD	This work
pVLAh126	pUC19 with a 3.6 kb insert from <i>A. hydrophila</i> 7966; expresses a MnSOD	This work
<i>E. coli</i>		
QC868	F ⁻ <i>leu6 thrA1 pro lacY1 supE44 hsdR</i> (r ⁻ m ⁺) <i>rpsL</i> (Sm ^R) <i>tonA1 thi1</i>	D. Touati
QC871	F ⁻ <i>leu6 thrA1 pro lacY1 supE44 hsdR</i> (r ⁻ m ⁺) <i>rpsL</i> (Sm ^R) <i>tonA1 thi1 sodA25 sodBA2</i> (Cm ^R Kan ^R)	D. Touati
EcA125	QC871 derivative carrying pVLAh125	This work
EcA126	QC871 derivative carrying pVLAh126	This work
<i>A. hydrophila</i>	ATCC 7966	ATCC

Table 2. Influence of PQ on bacterial growth

Growth was evaluated by measuring OD₆₀₀. See Table 1 for details of strains; Ah 7966, *A. hydrophila* ATCC 7966. 0, control (no PQ); PQ, 100 µM PQ added at OD₆₀₀ 0.2.

Culture period (h)	Ah 7966		QC868		QC871		EcA125		EcA126	
	0	PQ	0	PQ	0	PQ	0	PQ	0	PQ
6	3.18	3.08	2.04	2.07	1.11	0.24	1.73	1.49	1.72	1.51
24	4.56	4.99	4.84	5.01	4.55	0.35	3.58	3.48	3.31	3.37

Fig. 1. Expression of SOD activities encoded by *sod* genes from *A. hydrophila* and inhibition patterns. Lanes: A1, *E. coli* QC868; A2, *E. coli* QC871 (SOD⁻); B, EcA125; C, EcA126; D, *A. hydrophila* (Ah) ATCC 7966. Crude extracts were prepared from mid-exponential-phase (A1, A2, B, C) or late-stationary-phase (D) bacteria. All cells were grown in LB medium. (a) Gel stained for SOD activity; (b), (c) gels treated with 5 mM H₂O₂ or 2 mM KCN respectively.

growth measurements in the presence of 100 µM PQ (Table 2). *A. hydrophila* was naturally resistant to PQ at this concentration. Clones EcA125 and EcA126 showed the same PQ resistance as *E. coli* QC868, while *E. coli* QC871 (SOD⁻) did not grow under these conditions.

The ability of these clones to express SOD activity was tested on PAGE gels from crude cellular extracts. The mutants complemented by plasmids pVLAh125 and pVLAh126 each expressed a single SOD, while QC868 expressed two distinct activities and QC871, as expected, had no detectable SOD (Fig. 1a). The SODs expressed from EcA125 and EcA126 had different inhibition patterns and *R_F* values; these *R_F* values were 0.663 and 0.611 for *A. hydrophila* FeSOD and MnSOD respectively versus 0.583 and 0.265 for the corresponding enzymes of *E. coli* QC868 (Fig. 1b, c). Clone EcA125 produced an FeSOD as deduced by H₂O₂ inhibition and KCN resistance, whereas EcA126 synthesized a MnSOD as evidenced by resistance to both H₂O₂ and KCN.

Sequencing and analysis of the *sodA* and *sodB* genes

After sequencing of the 2.9 kb insert of the plasmid pVLAh125, a fragment of about 800 bp sharing high homology with *E. coli* *sodB* gene was suspected to bear

the promoter and the coding sequence for the FeSOD of *A. hydrophila*. The promoter region included some characteristic signals necessary for transcription: an initiation sequence (+1; AACAA), an AT-rich region (-10; TTATTT) and a -35 box (-33; TTTGGGC). An ATG preceded by a Shine-Dalgarno sequence (GGAGA) allowed the translation of a 194-residue protein with a theoretical pI of 6.14 and molecular mass of 21.5 kDa. The encoded protein shared 75% homology with the *E. coli* FeSOD (146/194 residues) including the amino acids implicated in the metal ligand binding (H₂₇-H₇₄ and D₁₅₈-H₁₆₂). A 12 bp inverted repeat sequence followed by a stretch of Ts was found 31 bp downstream of the stop codon and could function as a *rho*-independent RNA polymerase terminator.

The 3.6 kb insert of pVLAh126 contained a complete promoter and coding sequence (*sodA*) for a protein of 204 amino acids sharing 55% identity with *E. coli* MnSOD (114/206 residues). A transcription initiation sequence (+1; AACAA), an AT-rich region (-13; ATTAAT) and a Shine-Dalgarno box (+5; GAGG) were found upstream of the ATG (+14). Three potential -35 boxes (TTG^A/T^ACA) could also be found within the sequence, but the role of these sequences has not been studied. An inverted 11 nt repeated sequence centred on

Fig. 2. Kinetics of SOD expression during *A. hydrophila* growth. Crude extracts (10 µg total protein per lane) were prepared at different time points, corresponding to different growth stages. Cells were grown in LB medium under (a) high aeration or (b) low aeration. R, crude extract prepared from *A. hydrophila* grown for 48 h in LB medium under high aeration and used as reference.

Table 3. SOD activity in crude extracts of bacteria grown for 16 h in the presence of PQ

All the strains (see Table 1 for details) were grown in LB medium under high aeration, with PQ at the concentrations indicated. Values, expressed in U (mg total protein)⁻¹ are means ± standard deviations of triplicate assays.

Strain	PQ (µM):			
	0	10	10 ²	10 ³
<i>E. coli</i> QC868	3.86 ± 0.12	9.48 ± 0.54	20.8 ± 1.27	26.47 ± 1.64
<i>E. coli</i> EcA126	14.4 ± 1.84	13.28 ± 1.71	17.44 ± 2.21	21.87 ± 2.35
<i>A. hydrophila</i> ATCC 7966	6.75 ± 0.83	6.41 ± 0.73	5.38 ± 0.96	6.04 ± 1.15

nucleotide 661 with a probable hairpin structure could correspond to the *rho*-independent terminator sequence of the mRNA. The ORF of about 700 bp encoded a protein with a theoretical pI of 6.07 and a molecular mass of 22.3. Amino acids important for the ligand binding were conserved (G₇₇, G₇₈, F₈₅, Q₁₅₀ and D₁₅₁) and the 12 first amino acids constituted a potential signal peptide with the most likely cleavage site between A₁₁ and Y₁₂.

Growth-phase-dependent expression of the MnSOD

When *E. coli* QC868 was grown in LB medium under high aeration to mid-exponential phase, both FeSOD and MnSOD were detected on PAGE. EcA126 expressed the MnSOD under the same experimental conditions. A further assay, using *A. hydrophila* samples withdrawn hourly during 30 h, showed that total SOD activity from crude extracts remained unchanged: 5.92 ± 1.62 units SOD (mg total protein)⁻¹ at 3 h (mid-exponential phase) and 5.96 ± 1.15 units SOD mg⁻¹ at 16 h (stationary phase). On PAGE, the FeSOD was expressed whatever the growth phase whereas the MnSOD only appeared on gels after 16 h culture, corresponding to the stationary phase (Fig. 2a).

In other experiments using *A. hydrophila* grown under low aeration, samples were regularly assayed for SOD activity and loaded on PAGE gels. The SOD activity was

identical under either high or low aeration. However, only the FeSOD was continuously detectable in crude extracts from bacteria grown under low aeration during the 30 h incubation period, whereas the MnSOD was never detected even after 30 h (Fig. 2b).

Effect of iron deficiency on MnSOD synthesis

A. hydrophila was grown in LB medium in which iron was sequestered by either chemical (DIP, EDDA) or biological (Desferal) chelators. Whatever the chelator used, an identical result was obtained: the lack of iron induced the expression of MnSOD during mid-exponential phase, since the MnSOD was detectable on PAGE gels after 3 h growth (data not shown).

Effect of PQ on expression of the MnSOD

To test the induction of the *sodA* gene, *A. hydrophila*, *E. coli* EcA126 and *E. coli* QC868 (SOD⁺) were grown in the presence of different concentrations of PQ (10 to 10³ µM). PQ at any concentration did not affect the growth rate or the final biomass and the MnSOD was not detectable on PAGE gels before the stationary phase (data not shown). The total SOD activity assayed during stationary phase (16 h) remained unchanged for *A. hydrophila* and slightly enhanced for pVLAh126 cloned in *E. coli* QC871 (strain EcA126), whereas it was strongly stimulated for *E. coli* QC868 (Table 3).

Fig. 3. Subcellular localization of *A. hydrophila* MnSOD. Cells were fractionated after 48 h growth in LB medium under high aeration. R, crude extract; CF, cytosolic fraction; PF, periplasmic fraction.

Subcellular localization of the MnSOD

A. hydrophila cells were fractionated after 48 h growth in 100 ml LB medium under high aeration. The SOD activity measured was 5.18 ± 1.24 U (mg total protein) $^{-1}$ in the periplasmic fraction and 1.55 ± 0.52 U mg $^{-1}$ in the cytoplasmic fraction. For *E. coli* QC868, where the SODs are known to be cytoplasmic, the ratio between the two fractions was inverted (4.98 ± 0.32 U mg $^{-1}$ in the cytoplasmic fraction versus 13.18 U mg $^{-1}$ for the periplasmic space). PAGE of the *A. hydrophila* extracts showed that the MnSOD was distributed between the periplasmic and cytoplasmic fractions while the FeSOD was predominantly present in the cytoplasmic fraction (Fig. 3).

DISCUSSION

Two distinct SODs were identified in *A. hydrophila* ATCC 7966 and their complete genes were cloned. The sequenced insert of pVLAh125 contained an 800 bp fragment sharing high homology with the *sodB* gene from *E. coli* (Carlioz *et al.*, 1988). The protein exhibited 75% identity with the *E. coli* FeSOD, and the activity expressed corresponded to an FeSOD as determined by the inhibition patterns.

A positive effect of the iron-dependent regulatory Fur protein has been observed in the expression of the *sodB* gene of *E. coli* (Niederhoffer *et al.*, 1990) and recently the site of Fur regulation has been found in the promoter region. The site functioned as an extended -10 promoter containing a TGN sequence near the TA-rich region (Dubrac & Touati, 2000). Nevertheless the *A. hydrophila* FeSOD seemed to be constitutively expressed since the environmental changes such as iron limitation tested in this study did not affect its synthesis; moreover,

the *A. hydrophila* *sodB* promoter did not show the TGN sequence and so could be unaffected by the Fur regulator.

The sequenced insert of pVLAh126 contained a 700 bp gene encoding a MnSOD displaying 55% identity with the *E. coli* MnSOD (Takeda & Avila, 1986). The amino acids implicated in the metal binding, the Y₃₄ playing a catalytic role (Hunter *et al.*, 1997), and those pinpointed as potential discriminators between the iron and manganese proteins (G₇₇, G₇₈, F₈₅, N₁₄₉, Q₁₅₀, D₁₅₁ and V₁₈₉) (Parker & Blake, 1988), were conserved. A conservative change between the K₂₉ responsible for electrostatic steering of the substrate and an arginine residue was observed. The amino-terminal part of the protein (65 residues) shared high homology (71%) with that of *E. coli*. Nevertheless, although 9 out of the 12 first residues were conserved (MSHTLPLALYAY for *A. hydrophila* vs MSYTLPSLPYAY for *E. coli*), the *A. hydrophila* MnSOD contained a potential signal sequence lacking in *E. coli* Fe- and MnSODs (Nielsen *et al.*, 1997). A similar potential sequence suggesting a periplasmic location of the enzyme has been described in *Acinetobacter calcoaceticus* MnSOD (23 amino-terminal residues) (Geißdörfer *et al.*, 1997). In the fish pathogen *Aeromonas salmonicida* a periplasmic MnSOD has also been detected (Barnes *et al.*, 1996) and a role in the pathogenicity was proposed by the authors. This SOD may play a role in the defence against external reactive oxygen species like the periplasmic Cu/ZnSOD found in *E. coli* (Benov & Frodovich, 1996), *Legionella pneumophila* (Saint-John & Steinmann, 1996), *Haemophilus influenzae* and *H. parainfluenzae* (Kroll *et al.*, 1991, 1995). Recently, the molecular analysis of genetic differences between virulent and avirulent strains of *A. hydrophila* isolated from diseased fish did not indicate SODs as virulence factors (Zhang *et al.*, 2000).

As mentioned by Barnes *et al.* (1996) for *A. salmonicida*, the MnSOD of *A. hydrophila* was induced when no iron was available in the medium. In *E. coli*, *sodA* was regulated by the Fur repressor binding to iron boxes, consensus sequences NAT^A/TAT (Escobar *et al.*, 1998), present in the promoter. These iron-regulatory sequences were not found in the region 300 bp upstream of the *sodA* gene of *A. hydrophila* although a Fur protein was present in the wild-type *A. hydrophila* 495A2 (Barghouthi *et al.*, 1991). So it remains unclear whether the regulation is Fur-dependent, and further investigation is necessary to determine the mechanism of regulation of *sodA* by iron.

In LB medium, the *A. hydrophila* MnSOD was only expressed in the stationary phase under high aeration, even with an excess of iron (data not shown). These results are in accordance with the high expression of MnSOD in response to elevated oxygen levels and upon changes in growth phase in *E. coli* (Compan & Touati, 1993). Similarly increased levels of SOD activity during stationary phase were observed in other bacterial genera (Saint-John & Steinmann, 1996; Inaoka *et al.*, 1998; Clements *et al.*, 1999). The authors suggested that enhanced SOD levels are connected with survival of the bacterial cells under stressed conditions. The expression

of a MnSOD can also be related to quorum sensing, as was recently demonstrated for *Pseudomonas aeruginosa* (Bollinger *et al.*, 2001).

The *sodA* gene of *A. hydrophila* was not regulated by addition of PQ to the medium even in *E. coli* Eca126 although PQ enters the cells, supported by the fact that the expression was strongly stimulated by PQ in *E. coli* QC868. This non-regulation by PQ may not be so surprising considering the periplasmic location of the MnSOD in *A. hydrophila* and the increased flux of intracellular superoxide caused by PQ (Hassan, 1984). Physiological studies are necessary to determine the precise function of the MnSOD for *Aeromonas*.

Lastly, considering the particular conditions of the MnSOD expression in *A. hydrophila* described in this paper, together with the previously reported occurrence of this enzyme in *A. salmonicida* (Barnes *et al.*, 1996) it now appears clear that the presence of a unique SOD in *Vibrionaceae* should no longer be taken into account to distinguish this family from *Enterobacteriaceae*.

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REFERENCES

- Barghouthi, R., Payne, S. M., Arceneaux, J. E. L. & Byers, B. R. (1991). Cloning, mutagenesis, and nucleotide sequence of a siderophore biosynthetic gene (*amoA*) from *Aeromonas hydrophila*. *J Bacteriol* **173**, 5121–5128.
- Barnes, A. C., Horne, M. T. & Ellis, A. E. (1996). Effect of iron on expression of superoxide dismutase by *Aeromonas salmonicida* and associated resistance to superoxide anion. *FEMS Microbiol Lett* **142**, 19–26.
- Baumann, P. & Schubert, R. H. W. (1984). *Vibrionaceae*. In *Bergey's Manual of Systematic Bacteriology*, vol. 1, pp. 516–550. Edited by N. R. Krieg & J. G. Holt. Baltimore: Williams & Wilkins.
- Beauchamp, C. & Fridovich, I. (1971). Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Anal Biochem* **44**, 276–287.
- Benov, L. & Fridovich, I. (1996). Functional significance of the Cu,ZnSOD in *Escherichia coli*. *Arch Biochem Biophys* **327**, 249–253.
- Benov, L., Chang, L. Y., Day, B. & Fridovich, I. (1995). Copper, zinc superoxide dismutase in *Escherichia coli*: periplasmic localization. *Arch Biochem Biophys* **319**, 508–511.
- Bollinger, N., Hassett, D. J., Iglewski, B. H., Costerton, J. W. & McDermott, T. R. (2001). Gene expression in *Pseudomonas aeruginosa*: evidence of iron override effects on quorum sensing and biofilm-specific gene regulation. *J Bacteriol* **183**, 1990–1996.
- Carlioz, A., Ludwig, M. L., Stallings, W. C., Fee, J. A., Steinmann, H. M. & Touati, D. (1988). Iron superoxide dismutase: nucleotide sequence of the gene from *Escherichia coli* K12 and correlations with crystal structures. *J Biol Chem* **263**, 1555–1562.
- Clements, M. O., Watson, S. P. & Foster, S. J. (1999). Characterization of the major superoxide dismutase of *Staphylococcus aureus* and its role in starvation survival, stress resistance, and pathogenicity. *J Bacteriol* **181**, 3898–3903.
- Colwell, R. R., MacDonell, M. T. & De Ley, J. (1986). Proposal to recognize the family *Aeromonadaceae* fam. nov. *Int J Syst Bacteriol* **36**, 473–477.
- Compan, I. & Touati, D. (1993). Interaction of six global transcription regulators in expression of manganese superoxide dismutase in *Escherichia coli* K-12. *J Bacteriol* **175**, 1687–1696.
- Demple, B. (1991). Regulation of bacterial oxidative stress genes. *Annu Rev Genet* **25**, 315–337.
- Droillard, M. J., Bureau, D., Paulin, A. & Daussart, J. (1989). Identification of different classes of superoxide dismutase in carnation petals. *Electrophoresis* **10**, 44–68.
- Dubrac, S. & Touati, D. (2000). Fur positive regulation of iron superoxide dismutase in *Escherichia coli*: functional analysis of the *sodB* promoter. *J Bacteriol* **182**, 3802–3808.
- Escobar, L., Pérez-Martin, J. & de Lorenzo, V. (1998). Binding of the Fur (ferric uptake regulator) repressor of *Escherichia coli* to arrays of the GATAAT sequence. *J Mol Biol* **283**, 537–547.
- Fridovich, I. (1986). Superoxide dismutases. *Adv Enzymol* **58**, 61–97.
- Geißdörfer, W., Ratajczak, A. & Wolfgang, H. (1997). Nucleotide sequence of a putative periplasmic Mn superoxide dismutase from *Acinetobacter calcoaceticus* ADP1. *Gene* **186**, 305–308.
- Hassan, H. M. (1984). Determination of microbial damage caused by oxygen free radicals, and the protective role of superoxide dismutase. *Methods Enzymol* **105**, 404–412.
- Hunter, T., Ikebukuro, K., Bannister, W. H., Bannister, J. V. & Hunter, G. J. (1997). The conserved residue tyrosine 34 is essential for maximal activity of iron-superoxide dismutase from *Escherichia coli*. *Biochemistry* **36**, 4925–4933.
- Inaoka, T., Matsumura, Y. & Tsuchido, T. (1998). Molecular cloning and nucleotide sequence of the superoxide dismutase gene and characterization of its product from *Bacillus subtilis*. *J Bacteriol* **180**, 3697–3703.
- Kirov, S. M. (1997). *Aeromonas* and *Plesiomonas* species. In *Food Microbiology: Fundamentals and Frontiers*, pp. 265–287. Edited by M. P. Doyle, L. R. Beuchat & T. J. Montville. Washington, DC: American Society for Microbiology.
- Kroll, J. S., Langford, P. R. & Loynds, B. (1991). Copper-zinc superoxide dismutase of *Haemophilus influenzae* and *H. parainfluenzae*. *J Bacteriol* **173**, 7449–7457.
- Kroll, J. S., Langford, P. R., Wilks, K. E. & Keil, A. D. (1995). Bacterial [Cu,Zn]-superoxide dismutase: phylogenetically distinct from the eukaryotic enzyme, and not so rare after all! *Microbiology* **141**, 2271–2279.
- Leclère, V., Boiron, P. & Blondeau, R. (1999). Diversity of superoxide-dismutases among clinical and soil isolates of *Streptomyces* species. *Curr Microbiol* **39**, 365–368.
- Merino, S., Rubires, X., Knochel, S. & Tomas, J. M. (1995). Emerging pathogens: *Aeromonas* spp. *Int J Food Microbiol* **28**, 157–168.
- Niederhoffer, E. C., Najanro, C. M., Bradley, K. L. & Fee, J. A. (1990). Control of *Escherichia coli* superoxide dismutase (*sodA* and *sodB*) genes by the ferric uptake regulation (*fur*) locus. *J Bacteriol* **172**, 1930–1938.
- Nielsen, H., Engelbrecht, J., Brunak, S. & Von Heijne, G. (1997). Identification of prokaryotic and eukaryotic signal peptides and prediction of their cleavage sites. *Protein Eng* **10**, 1–6.
- 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.

Saint-John, G. & Steinmann, H. M. (1996). Periplasmic copper-zinc superoxide dismutase of *Legionella pneumophila*: role in stationary-phase survival. *J Bacteriol* **178**, 1578–1584.

Takeda, Y. & Avila, H. (1986). Structure and gene expression of the *E. coli* Mn-superoxide dismutase gene. *Nucleic Acids Res* **14**, 4577–4589.

Youn, H.-D., Kim, E.-J., Roe, J.-H., Hah, Y. C. & Kang, S.-O. (1996).

A novel nickel containing superoxide dismutase from *Streptomyces* spp. *Biochem J* **318**, 889–896.

Zhang, Y. L., Ong, C. T. & Leung, K. Y. (2000). Molecular analysis of genetic differences between virulent and avirulent strains of *Aeromonas hydrophila* isolated from diseased fish. *Microbiology* **146**, 999–1009.

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