A catalase from *Streptomyces coelicolor* A3(2)

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Catalase was purified from the Gram-positive bacterium *Streptomyces coelicolor* A3(2) in a three-step purification procedure comprising (NH₄)₂SO₄ fractionation, Phenyl-Sepharose chromatography and Mono Q chromatography. The purification of catalase, as judged by the final specific activity of 110 000 U mg⁻¹, was 250-fold with a 35% yield. The native protein was a homotetramer with a subunit *M*_r 55 000. N-terminal and internal peptide sequence analyses showed that there was a high degree of sequence similarity between the *S. coelicolor* catalase and other microbial and mammalian catalases. Southern blot analysis indicated that there was a single catalase gene in *S. coelicolor*. The specific activity of catalase throughout the growth of batch cultures was investigated and elevated catalase activity was found in stationary-phase cells.

Keywords: catalase, *Streptomyces coelicolor*

INTRODUCTION

Oxidative stress occurs when the concentration of active oxygen species in a cell is greater than the scavenging defence of the cell and damage to DNA, RNA, protein and lipids can occur. Consequently, virtually all aerobic organisms have evolved complex inducible deterrence and repair mechanisms to alleviate the damaging effects of active oxygen (McCord *et al.*, 1971; McCord & Fridovich, 1988).

One anti-oxidant mechanism, namely the scavenging of H₂O₂, is mediated by the activity of peroxidases and catalases. The most common form of catalase in both prokaryotes and eukaryotes is a haem protein which is active as a tetramer of subunits of 60 000 *M*_r, with a maximum of one protohaem IX group bound per subunit (Deisseroth & Dounce, 1970). Other divergent forms of differing subunit size, structure and prosthetic group have been identified in different bacteria, e.g. *Escherichia coli* (Loewen & Switala, 1986; Claiborne *et al.*, 1979) and *Bacillus subtilis* (Loewen & Switala, 1987, 1988). Many bacteria also possess a bifunctional catalase-peroxidase, with properties distinct from the classical catalases and peroxidases described from higher organisms. In *E. coli* this activity resides in a tetramer of subunit *M*_r 78 000 (Claiborne & Fridovich, 1979). The sequence of this

protein bears no resemblance to other catalases (Triggs-Raine *et al.*, 1988); instead it appears more related to the peroxidases of *Bacillus stearothermophilus* (Loprasert *et al.*, 1989) and *Mycobacterium tuberculosis* (Zhang *et al.*, 1992).

In many organisms catalase activity increases in response to oxidative stress (Jacob & Orme-Johnson, 1977; Christman *et al.*, 1985; Loewen *et al.*, 1985; Chary & Natvig, 1989; Lee *et al.*, 1993). In *E. coli* the bifunctional catalase-peroxidase, KatG, is inducible by H₂O₂ (Loewen *et al.*, 1985). Furthermore, catalase activity is also modulated by internal developmental events (Loewen & Switala, 1988; Chary & Natvig, 1989), and in a growth-phase-dependent manner as described for the *E. coli* monofunctional catalase, KatE (Schellhorn & Hassan, 1988).

The genus *Streptomyces* comprises a group of Gram-positive obligately aerobic bacteria. They exhibit a sedentary mycelial growth habit, followed by morphological differentiation to form spores which are dispersed to colonize new environments. This developmental stage is preceded by nutrient depletion, and often coincides with antibiotic production (Chater, 1984). Thus, antibiotic production can be thought of as a form of stress response (Demain, 1989). *Streptomyces coelicolor* A3(2) is a genetically well-characterized strain which provides a suitable vehicle for the study of stress responses, growth, antibiotic production and their possible relationships.

Little is known about the oxidative defences of *S. coelicolor*, although recent evidence has indicated that a coordinated response to oxidants does occur (Lee *et al.*, 1993). To

Abbreviations: FPLC, fast protein liquid chromatography; PVDF, polyvinylidene difluoride.

The PIR accession number for the peptide sequence reported in this paper is A44639.

elucidate one component of the oxidative defence mechanism, a study of catalase was undertaken in *S. coelicolor*. This paper describes the purification of a single, soluble catalase from *S. coelicolor* at different stages of the growth cycle, and reports the N-terminal and internal amino acid sequences of this single catalase. After this work was completed a paper describing the characterization of the major catalase from *S. coelicolor* ATCC 10147 was published (Kim *et al.*, 1994).

METHODS

Reagents. Phenyl-Sepharose CL-4B was supplied by Pharmacia. Pre-packed Mono Q₂ and Superose 12 columns were obtained from Pharmacia and utilized on a Pharmacia fast protein liquid chromatography (FPLC) system. Junlon PW110 was a gift from Honeywell and Stein, Wellington, UK. Polyvinylidene difluoride (PVDF) membrane (Problott) was obtained from Applied Biosystems. [γ -³²P]ATP and Hybond-N nylon filters were obtained from Amersham.

Bacterial strains and growth conditions. *S. coelicolor* JI3456 (SCP1^{8F}, SCP2⁺) was provided by Professor D. A. Hopwood, John Innes Institute, Norwich, UK. Cultures were grown at 30 °C on an orbital shaker at 200 r.p.m either in 1 l conical flasks containing 200 ml medium or in 2 l flasks containing 400 ml medium. The minimal medium comprised inorganic salts supplemented with 0.4% (w/v) glucose as sole carbon and energy source (Hobbs *et al.*, 1989). For enzyme purification from exponential-phase cells, a 200 ml starter culture was inoculated with spores washed from one fresh soya-mannitol slope and grown for 18 h. This culture was then used to inoculate 9 × 400 ml cultures (20 ml inoculum per 400 ml), which were grown for a further 24 h (total growth time of 42 h) and harvested when growing exponentially. Growth experiments were carried out using the same procedure for the starter culture, then individual 2 l flasks were harvested at appropriate time points. For enzyme purification from stationary-phase cells, a 200 ml starter culture was inoculated with spores washed from one fresh soya-mannitol slope and grown for 40 h. This culture was then used to inoculate 6 × 400 ml cultures (30 ml inoculum per 400 ml), which were grown for a further 22 h (total growth time of 62 h) and harvested when in stationary phase.

Catalase assay. Catalase was assayed by following the disappearance of peroxide spectrophotometrically at 240 nm as described by Beers & Sizer (1952). The assay cocktail contained 50 mM Bistrispropane/HCl pH 7.0, 18 mM H₂O₂ in a final volume of 1 ml. Assays were carried out at 25 °C with one unit of enzyme activity defined as the amount catalysing the conversion of 1 µmol substrate into product min⁻¹.

Protein estimation. Protein was determined by the method of Bradford (1976) or by a variation of the Lowry method with BSA as standard.

Purification of catalase from exponential-phase cells. Unless otherwise stated, all manipulations following cell breakage were done at 4 °C.

Step 1: Extraction and centrifugation. A 135 g (wet weight) batch of exponential-phase cells of *S. coelicolor* was suspended in 20 ml extraction buffer (100 mM Bistrispropane/HCl pH 7.5) and broken by two passages through a French pressure cell (950 p.s.i.; 6.55 MPa). This material was then centrifuged at 100 000 *g* for 1 h. Catalase was purified from the resulting cell-free extract.

Step 2: Salt fractionation with (NH₄)₂SO₄. Solid (NH₄)₂SO₄ was added to give 35% saturation (209 g l⁻¹). The mixture was

stirred for 20 min and the precipitated proteins were removed by centrifugation at 28 000 *g* for 20 min. The supernatant was adjusted to 65% saturation with solid (NH₄)₂SO₄ (199 g l⁻¹) and stirred for 20 min. The precipitated protein was collected by centrifugation at 28 000 *g* for 20 min and redissolved in 10 ml 50 mM Bistrispropane/HCl pH 7.5, 0.9 M (NH₄)₂SO₄ (buffer A).

Step 3: Hydrophobic-interaction chromatography on Phenyl-Sepharose. The redissolved protein from step 2 was loaded onto a Phenyl-Sepharose hydrophobic interaction column (bed volume 10 ml) equilibrated in buffer A. The column was washed with buffer A until the *A*₂₈₀ of the eluate was less than 0.1. Protein was eluted from the column with a 200 ml gradient of 0.9–0 M (NH₄)₂SO₄ in 50 mM Bistrispropane/HCl pH 7.5, collecting 5 ml fractions. These were assayed for catalase activity and those containing the highest activity were retained.

Step 4: Anion-exchange chromatography on Mono Q. The previously retained fractions were concentrated and dialysed against 500 ml 20 mM Bistrispropane/HCl pH 7.5 (buffer B). The procedure was then carried out at room temperature using a Pharmacia FPLC system. The dialysate was applied to an analytical Mono Q anion-exchange column (volume 1 ml) equilibrated in buffer B. Following washing with buffer B, protein was eluted with a gradient of 200–400 mM NaCl in 25 ml buffer B. Fractions (0.5 ml) were collected and assayed as before.

Purification of catalase from stationary-phase cells. Unless otherwise stated all manipulations following cell breakage were done at 4 °C. Steps 1 and 2 were carried out as described above.

Step 3: Anion-exchange chromatography on Mono Q. The precipitated protein was redissolved in 5 ml 50 mM potassium phosphate pH 7.0 (buffer C) and dialysed overnight against buffer C. The next two procedures were carried out at room temperature using a Pharmacia FPLC system. The dialysate was applied to an analytical Mono Q anion-exchange column (volume 1 ml) equilibrated in buffer C. Following washing with buffer C, protein was eluted with a gradient of Na₂SO₄ in buffer C. Fractions (0.5 ml) were collected and assayed as before.

Step 4: Anion-exchange chromatography on Mono Q. The previously retained fractions were dialysed against 500 ml buffer B. The dialysate was then applied to an analytical Mono Q anion-exchange column (volume 1 ml) equilibrated in buffer B. Following washing with buffer B, protein was eluted with a gradient of 200–400 mM NaCl in 25 ml buffer B. Fractions (0.5 ml) were collected and assayed as before.

SDS-PAGE. This was performed by the method of Laemmli (1970), with a 3% (w/v) stacking gel and a 10% (w/v) running gel. After electrophoresis, gels were stained for protein using Coomassie Brilliant Blue.

Determination of native *M_r*. The native *M_r* of the purified protein was estimated by gel permeation chromatography on a Superose 12 column mounted on a Pharmacia FPLC system. Standard proteins of known *M_r* were used to calibrate the column: *E. coli* shikimate dehydrogenase (*M_r* 30 000), pig heart malate dehydrogenase (*M_r* 70 000), pig muscle lactate dehydrogenase (*M_r* 140 000), rabbit muscle aldolase (*M_r* 160 000), bovine catalase (*M_r* 232 000) and horse apoferritin (*M_r* 440 000). A standard curve of peak elution volume (*V_e*) against *M_r* was constructed. The column was washed with 50 mM potassium phosphate pH 7.0, 150 mM NaCl at a flow rate of 0.2 ml min⁻¹ and 0.1 ml fractions were collected and assayed appropriately.

Protein and peptide microsequencing. Essentially homogeneous samples of catalase were subjected to SDS-PAGE in a 10% (w/v) gel and electroblotted onto PVDF membrane for N-terminal sequence analysis. Samples were also loaded onto an

18% (w/v) SDS gel and the protein was partially digested with V8 proteinase during electrophoresis (Cleveland *et al.*, 1977), and electroblotted. Intact protein and peptide fragments were microsequenced (Matsudaira, 1987) using an Applied Biosystems 477A protein sequencer. Further samples were digested to completion with clostripain. The resulting peptides were purified by reversed-phase HPLC and sequenced using a Milligen 6600 Prosequencer.

Protein sequence analysis. The obtained protein and peptide sequences were compared to the sequence data bases by using either the BLAST algorithm (Altschul *et al.*, 1990) or the sequence analysis programs of the University of Wisconsin Genetics Computer Group (Devereux *et al.*, 1984).

Design and synthesis of oligonucleotide probes. From the primary sequence data of catalase, degenerate oligonucleotides were designed as gene probes. An oligonucleotide (32mer, five degeneracies) was designed corresponding to the N-terminal sequence of residues 2–12 of catalase using the known streptomycete codon usage (Wright & Bibb, 1992). Similarly, the sequence of residues 2–10 of the clostripain-derived peptide was used and an oligonucleotide constructed (24mer, four degeneracies). These oligonucleotides were synthesized by Dr V. Math, Department of Biochemistry, University of Glasgow, with an Applied Biosystems model 380A DNA synthesizer.

Molecular biological methods. Total DNA of *S. coelicolor* was prepared essentially as described by Hopwood *et al.* (1985). Other molecular procedures were carried out as described by Sambrook *et al.* (1989). Genomic DNA digests were transferred to Hybond-N as described by Southern (1975). Radiolabelled oligonucleotide was prepared by incubating [γ - 32]ATP and oligonucleotide with T4 polynucleotide kinase. Filters were incubated with radiolabelled oligonucleotide as described by Binnie (1990).

Filters for hybridization experiments were prepared as follows: aliquots (5 μ g) of *S. coelicolor* genomic DNA were digested with a range of restriction enzymes, the products were separated by electrophoresis through a 0.8% (w/v) agarose gel and transferred to a nylon filter. Hybridization with the N-terminal oligonucleotide was carried out with the following conditions: the filter was incubated with probe for 90 min in 6 $\times$ SSC (1 $\times$ SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 0.5% (w/v) sodium pyrophosphate and 0.5% (w/v) SDS at 60 $^{\circ}$ C. Washing was done at 65 $^{\circ}$ C in 1 $\times$ SSC, 0.05% SDS. The filter was then stripped and re-probed with the internal oligonucleotide under identical conditions of salt and temperature.

RESULTS AND DISCUSSION

Purification of catalase

A single catalase has been isolated from exponential- and stationary-phase cells of *S. coelicolor*. The enzyme was prepared from exponential-phase cells as described in

Fig. 1. SDS-PAGE of catalase fractions eluted from Mono Q. Lanes: 1 and 5, M_r markers; 2, 5 μ g catalase; 3, 8 μ g catalase; 4, 5 μ g catalase. The gel was stained with Coomassie Brilliant Blue.

Methods. The purification of catalase as judged by the final specific activity of 110 000 U mg^{-1} was 250-fold with a 35% yield (Table 1). Catalase was estimated to constitute more than 95% of the protein of the most highly purified preparations (Fig. 1).

The absorption spectrum of the purified *S. coelicolor* catalase is shown (Fig. 2). The absorption maximum at 406 nm is characteristic of the protohaem IX group found in mammalian and other monofunctional catalases (Deisseroth & Dounce, 1970; Kim *et al.*, 1994). The A_{406}/A_{280} ratio of 0.82 is comparable with that observed for other purified bacterial catalases and demonstrates that the haem groups have not been lost during purification (Jones *et al.*, 1970; Jouve *et al.*, 1983; Kim *et al.*, 1994).

At no stage in our purification procedures did we resolve more than one catalase activity, and we concluded that a single catalase was present in *S. coelicolor*. Evidence for a single catalase gene in *Haemophilus influenzae* was reported recently (Bishai *et al.*, 1994), the sequence of which bears striking similarity to the amino acid sequences obtained from the purified *S. coelicolor* catalase (Fig. 3).

Table 1. Purification of *S. coelicolor* catalase from exponential-phase cells

Step	Total protein (mg)	Total activity (U)	Specific activity (U mg^{-1})	Yield (%)	Purification (-fold)
1. Crude	430	190 000	440	100	1
2. $(\text{NH}_4)_2\text{SO}_4$	146	104 000	710	54	1.6
3. Phenyl-Sepharose	6.36	80 000	12 600	42	29
4. Mono Q	0.6	66 000	110 000	35	250

Fig. 2. Absorption spectrum of purified catalase. A 0.5 ml fraction of the most highly purified material was made to a final volume of 1 ml with distilled H₂O. The spectrum was recorded versus a blank of 20 mM Bistrispropane buffer (pH 7.5).

Quaternary structure

The subunit M_r of the purified enzyme, determined under denaturing conditions by comparing electrophoretic mobility with the mobility of standard proteins of known M_r (Fig. 1), was $55\,000 \pm 2000$. Partially purified material was used for the gel-permeation chromatography. A single peak of catalase activity was eluted and the measured peak V_e corresponded to a native M_r of $195\,000 \pm 15\,000$.

These data indicate that the enzyme is tetrameric and in this respect resembles the mammalian catalases (e.g. Deisseroth & Dounce, 1970), and differs from the dimeric catalase found in *E. coli* (Loewen & Switala, 1986; Claiborne *et al.*, 1979).

Microsequencing and comparison of amino acid sequences among several catalases

The sequence of the first 26 amino acid residues of the N-terminus was determined (Fig. 3). Peptides for sequencing were also generated by *in situ* proteolysis with V8 proteinase. These were transferred onto PVDF membrane and 65 amino acids were sequenced from three peptides. Peptides 2 and 3 overlapped by seven amino acids (bold lettering in Fig. 3). A further sample was subjected to proteolysis by clostripain, and the resulting peptides were purified by reversed-phase HPLC, and one peptide yielded 13 amino acids of sequence (peptide 4, Fig. 3).

A high degree of similarity between the *S. coelicolor* amino acid sequences and other microbial and mammalian catalases was observed (Fig. 3). The N-terminal amino acid sequence of *S. coelicolor* catalase showed the greatest similarity to other prokaryotic catalases (Fig. 3), including the bromoperoxidase-catalase of *Streptomyces venezuelae*. This organism, unlike *S. coelicolor*, produces the chlorinated antibiotic chloramphenicol. The bromo-

1	PENNQKPLTTVAGAPVDPNQNSLTSG	26	Sco N-terminus
1	VT.G...E...A...E.A.	24	Sve (71%)
1	MSSNK...SW...G...M.A.	24	Bsu (58%)
8	SHLAATN...MGN...A...A.	33	Hin (58%)
1	MTDRRN...NQ.V.IG...M.A.	25	Lse (54%)
19	AAQKPDV...GG.N..G.KL...V.	44	Blc (50%)
1	MTNQ...NE.Q.WADN.H.Q.AA	23	Lsa (46%)
73	KGSENYA...NQ.VRIA.D...RA.	98	Eco (42%)

GNWDLVGNNTPVFFFRDPLKFFXL Sco peptide 1

129	L...R..PD.	152	Hin (83%)
119	..Y.I.....VN....PDI	142	Lsa (71%)
140	I..I..A.L.PSF	163	Blc (71%)
120	..Y.....IK.AI..PDF	143	Sve (67%)
120	..Y.I.....I..AI..PDF	143	Bsu (67%)
120	..Y.F....L...I..AI..PDV	143	Lse (62%)
199	..IF.....I..IQ.AH..PDF	222	Eco (58%)

LNRRNPDNYFADVE**QAATSP**ANVVPGRSFXPDRMLN Sco peptide 2/3

306	E.F.....S..A.S.L...IGAS....Q	340	Hin (69%)
297	E.I..E...SI...HF...IGPS..K..Q	331	Sve (63%)
297	..D...E...E...T...GTL...IDVS..K..Q	331	Bsu (63%)
307	V....E...L..D.S.MP..IEPS..K..Q	341	Blc (63%)
385	F..EN....H.GHI...LD.TN.PL.Q	419	Eco (60%)
296	..DE..T.N.E.IQEL.....L...IEAS..KL.Q	330	Lsa (51%)
297	I..ET.SVG.N.GVL...MLPSE..L.Q	331	Lse (51%)

GPMLLQDVWFLEK Sco peptide 4

27	..T.I...HL...	39	Bsu (69%)
36	..L.A..L.LN..	48	Hin (62%)
27	..V.V...QLL...	39	Sve (62%)
102	..T..E.FILR..	114	Eco (54%)
26	A.S.I..YQL...	38	Lsa (54%)
28	..T..E.YVLI..	40	Lse (54%)
47	..L.V...V.TDE	59	Blc (54%)

Fig. 3. Multiple alignment of the *S. coelicolor* N-terminal and peptide amino acid sequences with bovine liver catalase and other prokaryotic catalase sequences. All sequences are shown by the single-letter amino acid notation. The letter X represents amino acid residues unidentified during the micro-sequencing. The organisms and/or sources of sequences are: Blc, bovine liver catalase (Schroeder *et al.*, 1982); Bsu, *B. subtilis* (Bol & Yasbin, 1991); Eco, *E. coli* K12 (von Ossowski *et al.*, 1991); Hin, *H. influenzae* (Bishai *et al.*, 1994); Lsa, *Lactobacillus sake* (Knauf *et al.*, 1992); Lse, *Listeria seeligeri* (Haas *et al.*, 1991); Sco, *S. coelicolor* (this study); Sve, *S. venezuelae* (S. Facey, K. H. Van Pee & L. C. Vining, unpublished; accession no. X74791). The amino acid positions of the aligned regions of the catalases are numbered to indicate their position within the entire coding region. Dots represent identity with the *S. coelicolor* sequence. Bold lettering in the peptide 2/3 sequence indicates where these two peptides overlap. The overall identity with the *S. coelicolor* sequence is indicated in parentheses and the organisms are listed in order of decreasing identity.

peroxidase is responsible for the formation of carbon-halogen bonds in the presence of peroxides as well as possessing catalase activity and very low peroxidase activity (Knoch *et al.*, 1989).

The peptide sequences obtained also exhibited a high degree of similarity with other prokaryotic catalases (Fig. 3). Among the peptide sequences obtained, some residues involved in catalysis and haem-binding were found to be conserved (Fig. 3), namely Asn-8 of peptide 1 (catalysis), Phe-13 and Phe-21 of peptide 1 (distal side for haem-binding), Pro-6, Asp-18 and Met-20 of peptide 3 (proximal side for haem-binding) (Fita & Rossmann, 1985).

Fig. 4. Southern blot analysis of *S. coelicolor* genomic DNA digested with the restriction endonucleases *Sst*I (lane 1), *Bam*HI (lane 2) and *Sal*I (lane 3), or undigested (lane 4). The size of the single strongly hybridizing band for each lane is indicated. The filter was then stripped, re-probed with the internal oligonucleotide and the same hybridizing bands were identified.

S. coelicolor appears to possess a typical tetrameric catalase similar to those isolated from eukaryotic sources (Deisseroth & Dounce, 1970; Saeh & Kaplan, 1973; Jacob & Orme-Johnson, 1979) and some bacteria (e.g. Jouve *et al.*, 1983; Kim *et al.*, 1994). This sequence information agrees with other data showing the catalases to be a well-conserved group of proteins (Okada *et al.*, 1987; von Ossowski *et al.*, 1991), although bacterial catalases do appear to be more heterogeneous in sequence than eukaryotic catalases (von Ossowski *et al.*, 1993).

Southern blot analysis

Southern blot analysis using oligonucleotide probes designed against N-terminal and internal peptide amino acid sequences derived from the *S. coelicolor* catalase yielded a single strongly hybridizing signal (Fig. 4), suggesting that a single catalase gene is present in *S. coelicolor*. This result is consistent with the purification data, which indicated that a single catalase activity was present.

Fig. 5. Growth cycle and specific activity of catalase. ○, Total protein; ●, catalase specific activity. Catalase activity was determined independently six times and the mean specific activity plotted with the SD shown by error bars. The time was measured from the start of the primary culture.

Relationship between growth and catalase activity

S. coelicolor was grown in minimal medium and samples were taken at different times throughout the growth of a batch culture and analysed for protein content and catalase activity. The results demonstrated that during growth catalase activity increased slowly in mid- to late-exponential phase and by almost threefold upon entry into stationary phase (Fig. 5). Similar elevated catalase activities were found in stationary-phase cells whenever such extracts were prepared.

The relatively high levels of catalase in stationary-phase cells made its purification straightforward. A simplified purification scheme using $(\text{NH}_4)_2\text{SO}_4$ fractionation and two Mono Q chromatography steps gave homogeneous enzyme of specific activity $125\,000\text{ U mg}^{-1}$ in 22% yield (Table 2). The purification factor was only 38-fold compared with 250-fold from exponential-phase cells. The N-terminal sequence, subunit M_r , elution charac-

Table 2. Purification of *S. coelicolor* catalase from stationary-phase cells

Step	Total protein (mg)	Total activity (U)	Specific activity (U mg ⁻¹)	Yield (%)	Purification (-fold)
1. Crude	60	200 000	3330	100	1
2. $(\text{NH}_4)_2\text{SO}_4$	24	140 000	5800	70	1.6
3. Mono Q 1	2.22	62 000	28 000	42	8
4. Mono Q 2	0.35	44 400	125 000	22	38

teristics from Mono Q and absorption spectrum were indistinguishable from those of enzyme purified from exponential-phase cells, confirming that the same form of catalase had been isolated in both cases.

Similar increases in catalase activity on entering stationary phase have been described in other systems (see for example Dowds *et al.*, 1987). In *E. coli*, carbon-starved cells develop enhanced resistance to several forms of stress such as heat shock, oxidation, osmotic shock and starvation, with many proteins being induced (Matin, 1991). The induction of a large number of these proteins requires the putative sigma factor KatF (Lange & Hengge-Aronis, 1991; McCann *et al.*, 1991). One of these proteins, the KatE monofunctional catalase of *E. coli* is not inducible with H₂O₂, but is regulated in a growth-phase-dependent manner (Schellhorn & Hassan, 1988) by KatF (Loewen & Triggs, 1984). In the Gram-positive bacterium *B. subtilis*, gene expression is required upon entry into the stationary phase to facilitate transition to the non-growing state, the initiation of the sporulation process and the production of extracellular enzymes and antibiotics (Sonenshein, 1989). Alternative sigma factors are responsible for the expression of these stationary-phase genes (Helmann & Chamberlin, 1988), and an analogous system exists in *Streptomyces* (Westpheling *et al.*, 1985; Buttner *et al.*, 1988). It would be interesting to identify promoters upstream of the *S. coelicolor* catalase gene and determine how the catalase level is regulated. To this end, a clone containing part of the *S. coelicolor* A3(2) catalase gene has been obtained (G. E. Walker, unpublished).

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