Isolation and Expression of the catA Gene Encoding the Major Vegetative Catalase in Streptomyces coelicolor Müller

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We isolated the catA gene for the major vegetative catalase from Streptomyces coelicolor Müller. It encodes a polypeptide of 488 residues (55,440 Da) that is highly homologous to typical monofunctional catalases. We investigated catA expression by analyzing both catA mRNA and catalase activity. catA expression was increased by H₂O₂ treatment but did not increase during stationary phase. A putative catalase (CatB) cross-reactive with anti-CatA antibody appeared during stationary phase and in the aerial mycelium.

Catalase is an oxidoreductase which transfers two electrons to H₂O₂, decomposing it into O₂ and H₂O. Most of the catalases characterized so far can be classified into one of two types based on their enzymological properties (references 15 and references therein): monofunctional (typical) catalases and bifunctional catalase-peroxidases. Multiple catalases have been found in organisms such as Escherichia coli (5, 6), Bacillus subtilis (16), Streptomyces coelicolor (13, 14), Saccharomyces cerevisiae (19, 20), and Aspergillus nidulans (17). The role of each enzyme in different stages of the growth of these organisms is not well understood.

In S. coelicolor Müller, multiple catalases exist in late phases of growth in liquid culture or after the onset of differentiation on surface culture (4, 14). The major catalase (Cat4) is a typical catalase which consists of four identical subunits of 56 kDa and is expressed at a rather constant level throughout the different growth phases (14). A similar catalase has been reported in S. coelicolor A3(2) by Walker et al. (23). The involvement of H₂O₂ or reactive oxygen species in signaling for cell development has been suggested from studies in many systems (3, 10, 18). In this respect, the role of each catalase in growth and differentiation deserves systematic investigation. As an initiating effort toward this goal, this paper describes the isolation and characterization of the catA gene for the major vegetative catalase Cat4, whose expression is induced by H₂O₂ but not during the stationary phase of growth, unlike other major catalases in E. coli and B. subtilis (2, 12).

Cloning and sequence analysis of the catA gene. The compilation and alignment of amino acid sequences from typical monofunctional catalases enabled us to design PCR primer pairs with which to isolate a catalase gene from S. coelicolor Müller (ATCC 10147) (see Fig. 1). A single PCR product of 276 bp was produced whose deduced amino acid sequence closely matched the conserved amino acid residues of typical catalases. Genomic Southern hybridization with this PCR fragment revealed a single prominent band from each restriction digest tested. There was also a minor hybridizing band which might bear a related gene. We screened a phage library of S. coelicolor Müller DNA to isolate the clone carrying the template gene (catA) for the PCR product. A 4.5-kb insert from one phage clone containing the catA gene was further charac-

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coli EsrD promoters, it is most likely that the EsrD holoenzyme recognized this promoter in E. coli.

Regulation of catA gene expression by H2O2 and growth phase. The total catalase activity in S. coelicolor in the presence or absence of H2O2 was measured at different growth phases. As shown in Fig. 3A, compared with the early exponential phase culture, the total catalase activity increased about sevenfold when cells were cultured until stationary phase in YEME liquid medium (11). In rapidly growing cells, 100 mM H2O2 induced catalase activity twofold. The inducibility by H2O2 decreased as cells entered stationary phase. We resolved different catalases in cell extracts by native polyacrylamide gel electrophoresis and examined the expression of each catalase under the same conditions (Fig. 3B). We observed that Cat4 (CatA) is the major catalase in rapidly growing cells, is induced by H2O2, and increases during exponential culture for up to 20 h. At late exponential phase (40-h culture), Cat4 decreased slightly but a new activity (Cat5) appeared, contributing to the increase in total catalase activity (Fig. 3B, lane 5). During stationary phase, we observed an emergence of high-molecular-weight catalase activity (Cat1) as well as an increase in the levels of activities comigrating with Cat4 and Cat5 (Fig. 3B, lane 7). The emergence of at least two catalases other than CatA contributed to the increase in total catalase activity at later phases of growth. However, the quantitative assessment of each individual catalase cannot be accurately achieved from this type of activity staining analysis.

We next monitored the changes in catA expression by measuring both its RNA and its protein product (Fig. 4). Northern
analysis revealed a single mRNA species of 1.7 kb (Fig. 4A). This corresponds to the size of the \textit{catA} ORF and implies that the message is monocistronic. The level of \textit{catA} mRNA increased up to fourfold following \( \text{H}_2\text{O}_2 \) treatment during exponential phase (Fig. 4A). No full-length \textit{catA} mRNAs were detected at stationary phase. However, it was extremely difficult to obtain intact RNAs, from cells at this growth phase, probably because of increased level of RNases. We therefore tried to detect a portion of \textit{catA} mRNA near the 5\textsuperscript{\prime} end by \textit{S1} nuclease protection with the same probe used for the experiment shown in Fig. 3. The level of \textit{catA} transcript was assessed by Northern blot analysis (A) and \textit{S1} nuclease mapping (B). The amount of CatA polypeptide was estimated by Western blot analysis (C). Each sample contained 50 \( \mu \)g of RNA (A), 50 \( \mu \)g of RNA (B), or 50 \( \mu \)g of total protein (C). Northern analysis revealed a transcript of 1.7 kb, consistent with the predicted size of monocistronic \textit{catA} mRNA. In Western analysis, two weak cross-reacting bands were detected beside the major 55-kDa CatA protein; they are indicated as CatX (92 kDa) and CatB (87 kDa).

In Western blot analysis, we observed the emergence of a \( \sim 87\)-kDa polypeptide (CatB) that cross-reacted with anti-Cat4 antibody during the stationary phase of growth. This polypeptide is likely to correspond to one of the catalases enhanced in 100-h cultures, either Cat1 or the increased catalase comigrating with Cat4 (Fig. 3B). Another cross-reacting band (CatX) that is weaker than CatB was also observed. The significance of these bands in relation to total catalase activity is not clear at this point. Cells were also harvested from solid medium at stages of substrate, aerial, and sporulated mycelia and analyzed for CatA protein by Western blotting. The level of CatA did not change significantly, consistent with the behavior of \textit{catA} gene expression observed in liquid culture (data not shown).

The characteristics of the \textit{S. coelicolor} \textit{catA} gene product and its mode of regulation suggest that \textit{S. coelicolor catA} is different from vegetative catalases of \textit{E. coli} (HPI) and \textit{B. subtilis} (catalase 1) in gene regulation and/or biochemical aspects. \textit{E. coli} HPI is an \textit{rpoS}-controlled catalase-peroxidase whose level increases during stationary phase (5, 12). \textit{B. subtilis} catalase 1 is controlled by \textit{spo0A} and increases during stationary phase (2). Since the level of CatA gradually increases and is inducible by \( \text{H}_2\text{O}_2 \) during the exponential growth phase, it is likely that the transcriptional level of the \textit{catA} gene reflects the intracellular concentration of \( \text{H}_2\text{O}_2 \) and/or the specific growth rate. As suggested for HPI of \textit{E. coli}, it may play a major role in reducing the concentration of \( \text{H}_2\text{O}_2 \) inside the cell (9).

\textbf{Nucleotide sequence accession number.} The nucleotide sequence of the \textit{catA} gene has been deposited in the EMBL/GenBank/DDBJ database (accession no. X96981).
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