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Seigo Shima · Alexander Netrusov · Melanie Sordel
Michaela Wicke · Gudrun C. Hartmann
Rudolf K. Thauer

Purification, characterization, and primary structure of a monofunctional catalase from *Methanosarcina barkeri*

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Abstract *Methanosarcina barkeri* is a strictly anaerobic, cytochrome-containing, methane-forming archaeon. We report here that the microorganism contains a catalase, which was purified and characterized. The enzyme with an apparent molecular mass of 190 kDa was shown to be composed of four identical subunits of apparent molecular mass of 54 kDa. The heme-containing enzyme did not exhibit peroxidase activity, which indicates that it is a monofunctional catalase. This is substantiated by the primary structure, which is related to that of other monofunctional catalases rather than to that of bifunctional catalase-peroxidases. The enzyme showed an $[S]_{0.5V}$ for H_2O_2 of 25 mM and an apparent V_{max} of 200,000 U/mg; it was inhibited by azide ($[I]_{0.5V}$ = 1 μM) and cyanide ($[I]_{0.5V}$ = 5 μM) and inactivated by 1,2,4-aminotriazole. The activity was almost independent of the pH (between pH 4 and 10) and the temperature (between 15 °C and 55 °C). Comparison of the primary structure of monofunctional catalases revealed that the enzyme from *M. barkeri* is most closely related to the monofunctional catalase of *Dictyostelium discoideum*.

Key words Catalase · Cytochromes · Heme · *Methanosarcina* · Methanogenic archaea

Abbreviations *kat* Gene encoding monofunctional catalase

S. Shima (✉) · M. Sordel · M. Wicke · G. C. Hartmann
R. K. Thauer
Max-Planck-Institut für terrestrische Mikrobiologie
und Laboratorium für Mikrobiologie des Fachbereichs Biologie,
Philipps-Universität, Karl-von-Frisch-Straße,
D-35043 Marburg, Germany
e-mail: shima@mailer.uni-marburg.de,
url: http://www.uni-marburg.de/mpi,
Tel. +49-6421-178200; Fax +49-6421-178209

A. Netrusov
Microbiology Department, Moscow University, Moscow, Russia

Introduction

Catalases catalyze the conversion of H_2O_2 to O_2 and H_2O and thus protect the organisms from the oxidative effect of H_2O_2, which is generated in an autoxidation reaction from O_2. They are present in all aerobic organisms and in aerotolerant anaerobic organisms. Two types of heme catalases, which are phylogenetically not related, are known: the monofunctional catalases and the bifunctional catalase-peroxidases. The monofunctional catalases have been found in all three domains of life, whereas the presence of the bifunctional catalases appears to be restricted to bacteria and archaea [for literature, see Brown-Peterson and Salin (1995), Fraaije et al. (1996), Loewen (1996), Rocha and Smith (1997), and Terzenbach and Blaut (1998)].

Methanosarcina barkeri belongs to the methanogenic archaea (Thauer 1998). These are strictly anaerobic microorganisms that have previously been thought not to be aerotolerant, although there have been reports to the contrary (Zhilina 1972; Kiener and Leisinger 1983). Recently, evidence has been presented that methanogenic archaea from the hindgut of termites possess catalase-like activity (Leadbetter and Breznak 1996). The enzyme with the catalase-like activity was not identified.

The only catalases from archaea characterized to date are those from halophilic archaea. A monofunctional catalase and a bifunctional one have been purified from *Halobacterium halobium* (Brown-Petersen and Salin 1993, 1995), and a bifunctional catalase has been purified from *Haloarcula marismortui* (Cendrin et al. 1994). In the genome of the sulfate-reducing *Archaeoglobus fulgidus,* an open reading frame predicted to encode for a bifunctional catalase has been detected (Klenk et al. 1997). A respective open reading frame has not been found in the genomes of *Methanococcus jannaschii* (Bult et al. 1996) and *Methanobacterium thermoautotrophicum* (Smith et al. 1997). The genomes of the two methanogenic archaea also do not appear to contain a gene encoding for a monofunctional catalase. The apparent absence of heme catalase in these two methanogens is in accordance with the

finding that Methanococcales and Methanobacteriales lack cytochromes (Deppenmeier et al. 1996) and probably also the ability to synthesize heme (Bult et al. 1996; Smith et al. 1997). The primary structure of a catalase from an archaeon has not yet been reported.

Evidence has recently been presented that *Methanosarcina* species can survive in oxic paddy soils, indicating that they are aerotolerant. The viability of cell suspensions of the organism did not decrease when the suspensions were incubated under oxic conditions for 200 min at 20 °C (Fetzer et al. 1993). We report here that these cytochrome-containing methanogens possess a monofunctional catalase, which was purified and characterized and whose encoding gene was cloned and sequenced.

Materials and methods

Organism, culture conditions, plasmids, and phages

Methanosarcina barkeri strain Fusaro (DSMZ 804) was from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany). The archaeon was grown on 1% methanol at 37 °C as described by Karrasch et al. (1989). The cells were harvested anaerobically in the exponential phase at a cell concentration of 5 g (wet mass)/l. The cloning vector λZAP Express, the helper phage ExAssist, *Escherichia coli* strains XL1 Blue-MRF′ and XLOLR, and *Pfu* DNA polymerase were from Stratagene. Ampicillin, kanamycin, 1,2,4-aminotriazole, and the substrates for the peroxidase activity test (see below) were from Sigma. Synthetic oligonucleotides were obtained from MWG-Biotech. The digoxigenin labeling kit and the luminescent detection kit for nucleic acids were from Boehringer Mannheim. All DNA-modifying enzymes were from United States Biochemicals. The Thermo Sequenase fluorescent-labeled primer cycle sequencing kit with 7-deaza-dGTP, the ALF express DNA sequencer, FPLC equipment, FPLC columns, and standard proteins for SDS-PAGE and gel-filtration columns were from Amersham Pharmacia Biotech.

Purification of catalase

Catalase was purified from 5 g (wet mass) methanol-grown cells of *M. barkeri*. The cells were suspended in 14 ml 50 mM potassium phosphate (pH 7.0). The cell suspension was passed three times through a French pressure cell at 110 MPa. Cell debris was removed by centrifugation for 20 min at 20,000 × *g* at 4 °C. The cell extract was centrifuged at 240,000 × *g* and 4 °C for 60 min. Ammonium sulfate powder was added to the supernatant to a final concentration of 55% saturation. The solution was incubated at 0 °C for 10 min and was centrifuged at 25,000 × *g* and 4 °C for 15 min. Then ammonium sulfate powder was added to the supernatant to a final concentration of 65% saturation. The solution was incubated at 0 °C for 10 min and was centrifuged at 25,000 × *g* and 4 °C for 15 min.

The precipitated fraction was dissolved in 12 ml of 1 M ammonium sulfate in 50 mM Mops-KOH (pH 7.0). The solution at 4 °C was applied to a Phenyl Sepharose 26/10 column that was equilibrated with 1 M ammonium sulfate in 50 mM Mops-KOH (pH 7.0). Catalase was eluted from the column with 250 ml of a linear decreasing gradient of ammonium sulfate (1–0 M). The flow rate was 4 ml/min. Fractions of 8 ml were collected. Catalase activity was eluted at 0.56–0.24 M ammonium sulfate. The fractions containing catalase activity were combined and concentrated by filtration (30-kDa cut-off) (Amicon) and diluted with 50 mM citric acid/NaOH (pH 5.3). The solution was applied to a Resource Q column (6-ml volume) that was equilibrated with 50 mM citric acid/NaOH (pH 5.3). Catalase did not bind to the column, and the activity was recovered in the flow through fractions. The catalase fraction was concentrated by filtration (30-kDa cut-off) and diluted with 50 mM Mops-KOH (pH 7.0). The solution was applied to a Resource Q column (6-ml volume) that was equilibrated with 50 mM Mops-KOH (pH 7.0). Catalase was eluted from the column with 100 ml of a linear increasing gradient of NaCl (0–0.2 M). The flow rate was 3 ml/min. Fractions of 4 ml were collected. Catalase activity was eluted at 0.11–0.16 M NaCl. The fractions containing catalase activity were combined and concentrated by filtration (30-kDa cut-off) and diluted with 10 mM potassium phosphate (pH 7.0). The solution was applied to a Ceramic Hydroxyapatite column (1.3 cm × 10 cm) (BioRad) that was equilibrated with 10 mM potassium phosphate (pH 7.0). Catalase was eluted from the column with 130 ml of a linear increasing gradient of potassium phosphate (10–500 mM). The flow rate was 3 ml/min. Fractions of 4 ml were collected. Catalase activity was eluted at 180–245 mM potassium phosphate (pH 7.0). The fractions containing catalase activity were combined and concentrated by filtration (30-kDa cut-off) and diluted with 10 mM Mops-KOH (pH 7.0). The solution was further concentrated by a Centricon 30 microconcentrator (Amicon) to 120 µl and was applied to a Superdex 200 column (1 cm × 30 cm) that was equilibrated with 10 mM Mops-KOH (pH 7.0). The flow rate was 0.5 ml/min. Fractions of 1 ml were collected. The fractions containing catalase activity were combined and concentrated to 100 µl by a Centricon 30 microconcentrator.

The enzyme was routinely stored at 4 °C. Under this condition, the catalase activity remained constant for at least 1 month.

Molecular properties of catalase

Analytical gel filtration was performed by chromatography on a Superdex 200 (1 cm × 30 cm) column that was equilibrated with 50 mM potassium phosphate (pH 7.0). The column was calibrated by standard protein solution containing ferritin (440 kDa), catalase (bovine liver; 232 kDa), aldolase (158 kDa), ovalbumine (43 kDa), and ribonuclease A (13.7 kDa).

The sequence of the catalase purified from *M. barkeri* was determined on a 477 A protein/peptide sequencer from Applied Biosystems by Dr. D. Linder (University of Gießen, Germany).

Determination of enzyme specific activity

Catalase activity was routinely determined spectrophotometrically at 25 °C by following the decrease in absorbance at 240 nm (ε_{240} nm = 39.4 M^{-1} cm^{-1}) of 13 mM H_2O_2 in 50 mM Tris-HCl (pH 8.0) (Beers and Sizer 1952; Nelson and Kiesow 1972). One unit (U) is defined as the amount of enzyme that catalyzes the oxidation of 1 µmol H_2O_2 min^{-1} under the assay condition. Peroxidase activity was tested spectrophotometrically at 25 °C in 50 mM Tris-HCl (pH 8.0) with 1.0 mM H_2O_2 and either 0.2 mM NADH, 0.2 mM NADPH, 0.2 mM ascorbate, 2.0 mM Dopa (L-3,4-dihydroxyphenylalanine), or 2.0 mM guaiacol.

Protein concentrations were determined by the method of Bradford (1976) using the reagents from BioRad and bovine serum albumin as standard.

Generation of a hybridization probe for the *kat* gene

A probe for the *kat* gene was obtained by PCR using genomic DNA from *M. barkeri* as template, which was isolated as described by Künkel et al. (1998). The heterologous oligonucleotides 5′-AC(A/G/C/T)GG(A/G/C/T)TT(C/T)GG(A/G/C/T)AT(A/C/T)CC (sense) and 5′-CC(A/G/T)GC(A/G/T)GT(A/G/C)AGG(G/C)(A/T)(A/G) TT(C/T)TG (antisense) were derived from the N-terminal amino acid sequence of purified catalase. The 25-µl PCR mixture contained 2.5 ng genomic DNA of *M. barkeri*, 1.3 U *Pfu* DNA polymerase, 100 µM dNTP, 2.0 mM $MgCl_2$, and 500 pM of each

of the two primers. The temperature program was 1 × 5 min at 95 °C, 30 cycles for 30 s at 94 °C/1 min at 45 °C, and 1 × 5 min at 72 °C. The 50-bp PCR product was amplified and cloned into the pCR-Blunt vector using the Invitrogen Zero Blunt PCR Cloning Kit. The identity of the cloned fragment was determined by DNA sequencing. The following 26-b oligonucleotide probe was designed from the PCR product: 5′-GGAATACCGGTGGGGATGATCAGAA, GC% = 53.8. The oligonucleotide was subjected to 3′-labeling with digoxigenin-dUTP following a protocol given by Boehringer Mannheim and then was used for Southern hybridizations (Sambrook et al. 1989) of *M. barkeri* genomic DNA digested to completion with restriction endonucleases. As a control, hybridization was also performed with a 3′-digoxigenin-dUTP-labeled 26-b oligonucleotide probe, 5′-GCACGGGTACTCATAACAGCTGCTAC, GC% = 53.8, which was derived from the sequence of the formyltransferase gene of *M. barkeri* (Kunow et al. 1996). The hybridization and washing conditions employed were 15 mM sodium citrate (pH 7.0) containing 150 mM NaCl at 55 °C.

Cloning and sequencing of the *kat* gene

The 26-b probe for *kat* was used to screen the λZAP Express *Sau*3AI genomic library of *M. barkeri* (Vorholt et al. 1996). *Escherichia coli* cells were infected with packaged λZAP DNA and were plated onto Luria-Bertani agar (Sambrook et al. 1989). Positive clones were identified by plaque hybridization. Excision and recircularization of pBK-CMV and its insert generated plasmid clones. A DNA fragment cloned into pBK-CMV vector was sequenced using the dideoxynucleotide method with fluorescent labeled primers.

Results

Cell extracts of *M. barkeri* were found to catalyze the disproportionation of H_2O_2 to O_2 and H_2O, as determined photometrically by following the decrease in H_2O_2 concentration at 240 nm. Per milligram of cell extract protein, approximately 40 μmol of H_2O_2 was diproportionated per minute under the standard assay conditions, which were 25 °C, pH 8.0, and 13 > mM H_2O_2. The catalase activity was associated with the soluble cell fraction. It was not sensitive to air. Therefore, the enzyme could be purified under oxic conditions.

Table 1 Purification of catalase from *Methanosarcina barkeri*. Cell extract was prepared from 5 g (wet mass) of methanol-grown *M. barkeri* and catalase was purified as described in Materials and methods (*1 U* = 1 μmol/min)

Fraction	Protein (mg)	Activity (U)	Specific activity (U/mg)	Purification (-fold)	Yield %
240,000 × *g* supernatant	263	10,500	40	1	100
55–65% $(NH_4)_2SO_4$ supernatant	215	16,300	76	1.9	155
Phenyl Sepharose HiLoad (26/10)	20	7,300	365	9.1	70
Resource Q (pH 5.3)	9.7	4,900	505	12.7	47
Resource Q (pH 7.0)	1.7	4,300	2,530	63.3	41
Hydroxyapatite	0.10	2,300	23,000	575	22
Superdex 200	0.026	1,600	61,500	1538	15

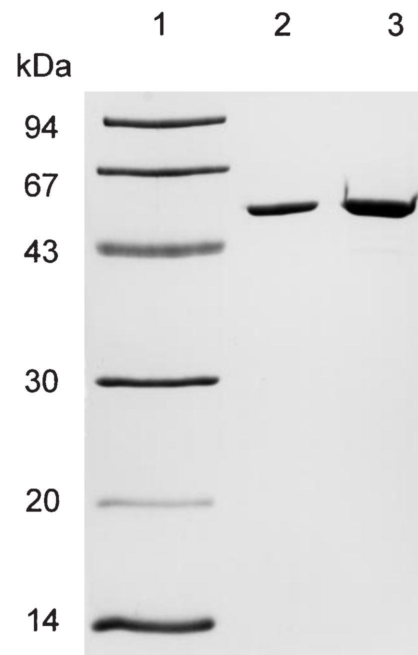


Fig. 1 SDS/PAGE of purified catalase from *Methanosarcina barkeri*. Protein was separated on a 12% polyacrylamide gel and subsequently stained with Coomassie brilliant blue. *Lane 1* low molecular mass standards (Amersham Pharmacia Biotech), *lane 2* 1 μg purified catalase, and *lane 3* 2 μg purified catalase

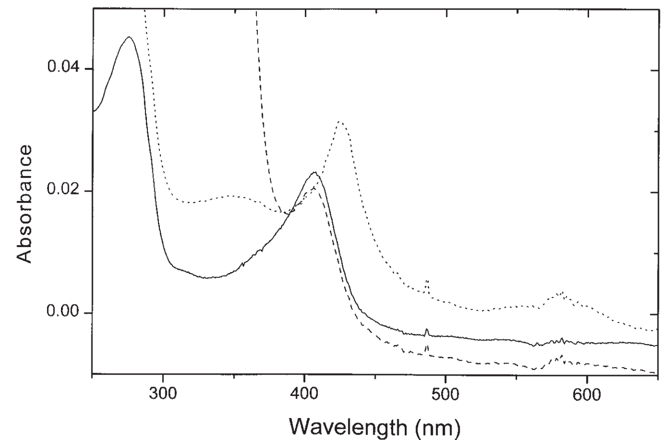


Fig. 2 Ultraviolet/visible spectrum of purified catalase from *Methanosarcina barkeri*. The solution was 5 μg enzyme in 50 μl 50 mM potassium phosphate (pH 7.0). —— Without additions, – – – after reduction with 1 mM dithionite, and · · · · after addition of 10 mM KCN. The spectra were recorded with a Zeiss Specord S10 diode array spectrophotometer; the light path was 0.3 cm

Purification

The catalase activity was purified in six steps over 1,500-fold in a 15% yield (Table 1). From 263 mg cell extract protein, 0.026 mg purified enzyme with a specific activity of 61,500 U/mg was obtained. SDS-PAGE revealed the presence of only one major band at an apparent molecular mass of 54 kDa (Fig. 1). A minor band at an apparent mol-

Table 2 Molecular and catalytic properties of purified catalase from *Methanosarcina barkeri*

Properties	
Apparent molecular mass	190 kDa (gel filtration) 54 kDa (SDS/PAGE)
N-Terminal sequence	GEKNSSKVLTTGFGIPVG(D)(D) QNSLTAGN(?)GPV(L)MQ[35]
pI	6.3
Prosthetic group	Ferric heme
$[S]_{0.5V}$ for H_2O_2	25 mM
Apparent V_{max}	200,000 U/mg
$[I]_{0.5V}$ for KCN	5 µM
$[I]_{0.5V}$ for NaN_3	1 µM
$[I]_{0.5V}$ for 1,2,4 aminotriazole	80 mM at 25 °C for 60 min
pH dependence	Independent between pH 4 and 10
Temperature dependence	Independent between 15 °C and 55 °C
Temperature stability	Stable up to 55 °C
Peroxidase activity	None

ecular mass of 43 kDa was only visible when the gels were loaded with high amounts of protein (Fig. 1, lane 3) and is considered to be a contaminant since the intensity of the band, which varied from preparation to preparation, did not correlate with the specific catalase activity.

Molecular properties

The purified catalase eluted from gel-filtration columns (Superdex 200) with an apparent molecular mass of 190 kDa (not shown). Under denaturating conditions the protein migrated with an apparent molecular mass of 54 kDa (Fig. 1). It is therefore proposed that the catalase is a homotetrameric enzyme.

The ultraviolet/visible spectrum of the purified catalase indicated the presence of a heme prosthetic group (Fig. 2). The native enzyme had a Soret band at 407 nm. This band was shifted to 426 nm upon addition of cyanide. The prosthetic group was not reducible by dithionite. These are characteristic properties of monofunctional catalases (Brown-Peterson and Salin 1995; Terzenbach and Blaut 1998). The A_{407}/A_{280} ratio of 0.48 is low for typical catalases (which usually exhibit ratios of approximately 1) and may indicate that the heme was partially lost during the purification process.

The N-terminal amino acid sequence of the purified catalase (Table 2), which was determined by Edman degradation, showed significant sequence similarity (66% identity and 83% similarity) to the N-terminal amino acid sequence of the monofunctional catalase from *Bacillus subtilis*.

Catalytic properties

The activity of the purified catalase was routinely assayed at an H_2O_2 concentration of 13 mM. When the concentration was increased, the specific activity increased from 61,500 U/mg to approximately 200,000 U/mg at 50 mM H_2O_2. The concentration dependence between 6.5 and 40 mM followed the Michaelis-Menten equation: a plot of 1/v vs 1/[S] was linear (results not shown). At H_2O_2 concentrations higher than 50 mM, the rate no longer increased. Fifty percent of the maximal activity was observed at an H_2O_2 concentration of approximately 25 mM (Table 2). The specific activity of the catalase was almost pH-independent between pH 4 and 10 and temperature-independent between 15 and 55 °C. The latter property is typical for catalases (Brown-Peterson and Salin 1995).

The catalase activity was inhibited by cyanide and azide, half-maximal inhibition being observed at concentrations of 5 and 1 µM, respectively (Table 2). The enzyme was found to be inactivated by 1,2,4-aminotriazole ($[I]_{0.5V}$ = 80 mM at 25 °C for 60 min), which is a specific inhibitor of monofunctional catalases (Brown-Peterson and Salin 1995).

The purified catalase did not catalyze the oxidation of NADH, NADPH, ascorbate, Dopa (L-3,4-dihydroxyphenylalanine), and guaiacol by H_2O_2 and, thus, did not possess peroxidase activity (Fraaije et al. 1996; Pütter and Becker 1983).

Catalase-encoding gene

From the N-terminal amino acid sequence of the purified catalase (Table 2), two heterologous PCR primers were deduced to obtain a homologous probe for the *kat* gene encoding the catalase. With the probe, a *Sau*3AI gene bank from *M. barkeri* was screened. From 6,500 plaques, five were positive. From one of the positive plaques, the gene with its flanking regions was cloned and sequenced. The nucleotide sequence is available under accession no. AJ005939 in the EMBL database.

The *kat* gene starts with an ATG codon, stops with a TAA codon, and is 1,515 bp long. The DNA G+C content was 44 mol% and, thus, a little higher than the average G+C content of 42 mol% of the *M. barkeri* genome (Boone et al. 1993). The usage of the codons for arginine, asparagine, histidine, phenylalanine, and tyrosine differed significantly from that of other known genes in *M. barkeri* (Bokranz and Klein 1987; Harms and Thauer 1996; Kunow et al. 1996; Vorholt et al. 1996; Sauer et al. 1997; Vaupel and Thauer 1998; Vaupel et al. 1998).

The nucleotide sequence predicts that the catalase should be composed of 505 amino acids and should have a mass of 57,065 Da, which has to be compared with the apparent molecular mass of 54 kDa determined for the catalase subunit by SDS-PAGE. The N-terminal amino acid sequence deduced from the nucleotide sequence indicated the absence of the N-terminal methionine. The determined N-terminus started with GEKNSS, whereas the gene-deduced N-terminus started with MGEKNSS, indicating post-translational processing. From the amino acid sequence, a pI of 6.3 was calculated.

The sequence 10 bp upstream of the *kat* gene showed a ribosome binding site (AGGAG), and the sequence 22 bp

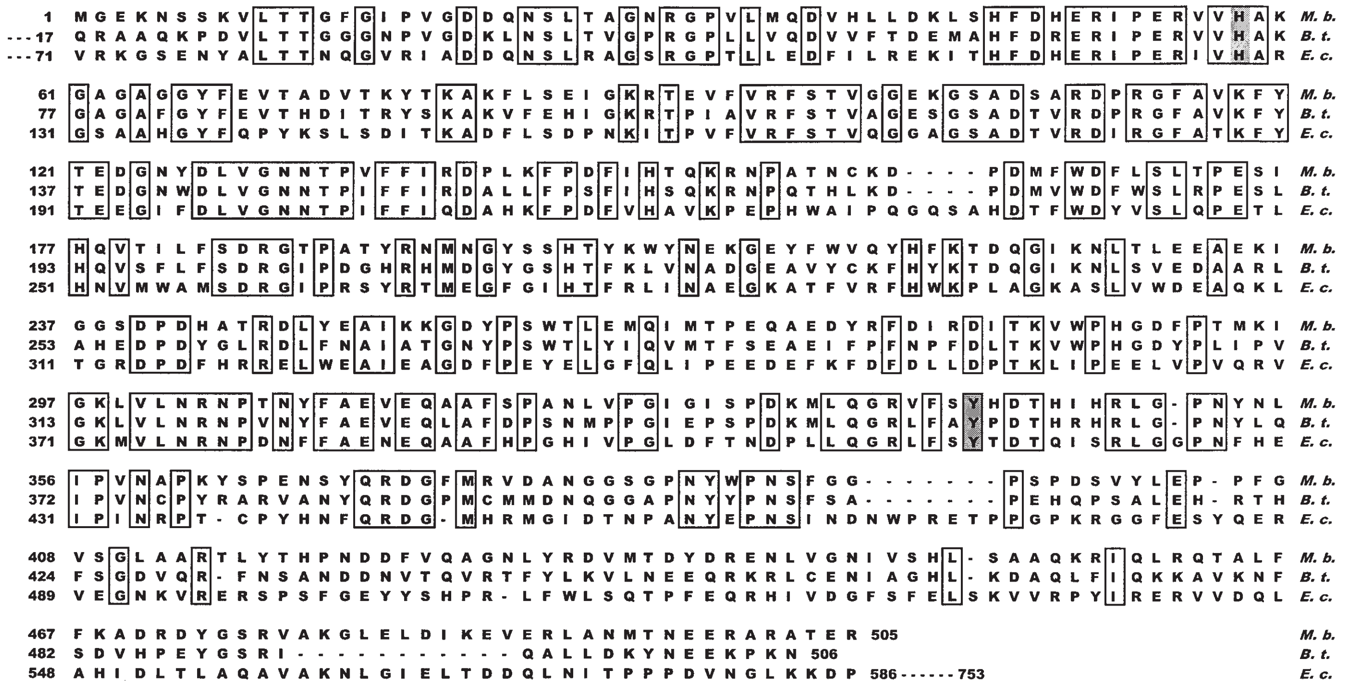


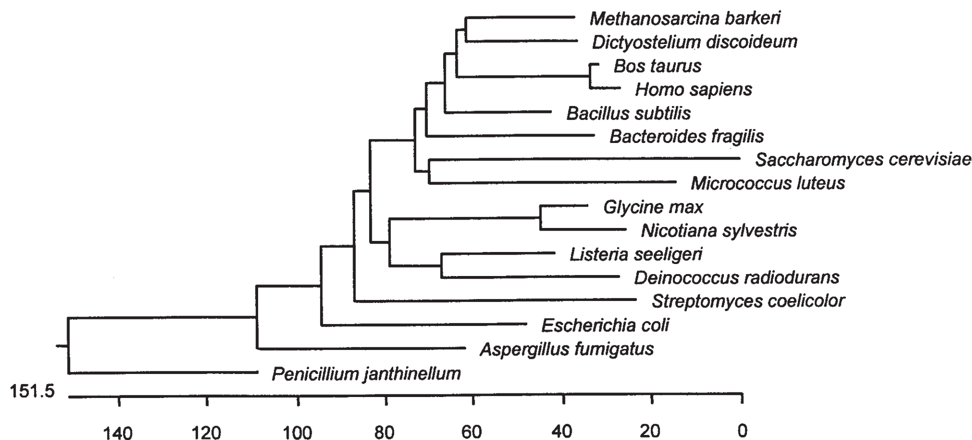
Fig. 3 Alignment of the deduced amino acid sequence of *Methanosarcina barkeri* catalase with sequences of a mammalian (*Bos taurus*) and a bacterial (*Escherichia coli*) catalase. Conserved amino acid residues are *boxed*. From the X-ray structure of the *B. taurus* catalase (Fita and Rossmann 1985) and of the *E. coli* catalase (Loewen 1996) it is known that Tyr-341 is the proximal ligand to the heme iron and that the imidazole ring of His-58 is situated immediately above ring III of the heme in a location where it participates as a catalytic factor (Loewen 1996) (*M. b., Methanosarcina barkeri*, *B. t., Bos taurus*, and *E. c., Escherichia coli*)

Fig. 4 Phylogenetic tree of catalases deduced from a comparison of the primary structure. The branching order was obtained from alignments using the Clustal method of the Megalign program (DNAStar, London, England). The units at the bottom of the tree indicate the number of substitution events. Accession numbers: AF090443 (*Dictyostelium discoideum*), P00432 (*Bos taurus*), P04040 (*Homo sapiens*), P26901 (*Bacillus subtilis*), P45737(*Bacteroides fragilis*), P06115 (*Saccharomyces cerevisiae*), P29422 (*Micrococcus luteus*), AF035255 (*Glycine max*), U07626 (*Nicotiana sylvestris*), A40367 (*Listeria seeligeri*), D63898 (*Deinococcus radiodurans*), AF000419 (*Streptomyces coelicolor*), P21179 (*Escherichia coli*), U97574 (*Aspergillus fumigatus*), and P81138 (*Penicillium janthinellum*)

downstream of the stop codon showed an inverted repeat followed by an oligo T sequence (TTTATTTT) that is probably a stop signal (Brown et al. 1989; Reeve 1992). The sequence AAATCTATTTATA 103 bp upstream of the translation initiation codon ATG could direct transcription initiation. The consensus sequence of promoters from methanogenic archaea is AAAXXTTTATATA (Brown et al. 1989; Reeve 1992).

The sequence was determined 235 bp upstream and 93 bp downstream of the *kat* gene. No open reading frames were found, which indicates that the *kat* gene is probably monocistronically transcribed.

With the homologous probe used to screen for the *kat* gene, Southern hybridizations were performed. In each restriction digest of genomic DNA, only one band was observed, indicating that the genome of *M. barkeri* harbors only one *kat* gene. As a control, the restriction digests were also probed with an oligonucleotide specific for the formyltransferase gene, which encodes for an enzyme involved in methanogenesis (Kunow et al. 1996; Thauer



1998). The intensity of the band was the same as that obtained with the homologous probe for the *kat* gene.

Discussion

M. barkeri was shown to contain catalase at a specific activity of 40 U/mg, which is in the same range as in the case of other aerotolerant anaerobes. It has been reported that the specific activity of catalase in *Methanobrevibacter cuticularis* from the hindgut of a termite is 54 U/mg (Leadbetter and Breznak 1996), and that in *Bacteroides fragilis* is 35.6 U/mg (Rocha and Smith 1995). Evidence is available that catalase protects *B. fragilis* from oxygen toxicity (Rocha et al. 1996).

The catalase from *M. barkeri* is a monofunctional catalase rather than a bifunctional catalase-peroxidase, as indicated by the following findings:

1. Most importantly, the enzyme did not exhibit peroxidase activity, and its primary structure showed similarity to that of monofunctional catalases and not to that of catalase-peroxidases.
2. Other features shared with monofunctional catalases are the inactivation of the enzyme by 1,2,4-aminotriazole, the non-reducibility of the heme prosthetic group with dithionite, and the constant activity between pH 4 and 10. The inhibition of the enzyme by azide and cyanide is a property also exhibited by bifunctional catalases.

The crystal structures of several monofunctional catalases have been solved (Fita and Rossmann 1985; Gouet et al. 1995; Loewen 1996). In these structures, the essential residues in the active site are conserved. They are also conserved in the primary structures of all other monofunctional catalases (Rocha and Smith 1995) including the catalase from *M. barkeri* (Fig. 3).

The primary structure of the catalase deduced from the nucleotide sequence from *M. barkeri* shows the highest sequence similarity to the primary structure of the monofunctional catalase from *Dictyostelium discoideum*, *Bos taurus*, *Homo sapiens,* and *Bacillus subtilis* (Fig. 4). The phylogeny of the catalase sequences does not follow the phylogeny based on 16S rRNA sequences. It has recently been proposed that the catalase gene sequence has migrated repeatedly from eukaryotes to prokaryotes (Mayfield and Duvall 1996). In this respect it is of interest that the *kat* gene in *M. barkeri* differs in DNA G+C content and codon usage from other genes in this organism.

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