The catalase and superoxide dismutase genes are transcriptionally up-regulated upon oxidative stress in the strictly anaerobic archaeon *Methanosarcina barkeri*

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Methanosarcina barkeri is a strictly anaerobic methanogenic archaeon, which can survive oxidative stress. The oxidative stress agent paraquat (PQ) suppressed growth of *M. barkeri* at concentrations of 50–200 µM. Hydrogen peroxide (H₂O₂) inhibited growth at concentrations of 0.4–1.6 mM. Catalase activity in cell-free extracts of *M. barkeri* increased about threefold during H₂O₂ stress (1.3 mM H₂O₂, 2–4 h exposure) and nearly twofold during superoxide stress (160 µM PQ, 2 h exposure). PQ (160 µM, 2–4 h exposure) and H₂O₂ (1.3 mM, 2 h exposure) also influenced superoxide dismutase activity in cell-free extracts of *M. barkeri*. Dot-blot analysis was performed on total RNA isolated from H₂O₂- and PQ-exposed cultures, using labelled internal DNA fragments of the *sod* and *kat* genes. It was shown that H₂O₂ but not PQ strongly induced up-regulation of the *kat* gene. PQ and to a lesser degree H₂O₂ induced the expression of superoxide dismutase. The results indicate the regulation of the adaptive response of *M. barkeri* to different oxidative stresses.

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INTRODUCTION

Methanosarcina barkeri belongs to the methanogenic archaea, very ancient micro-organisms which play a major role in the global production of methane as their end product of energy metabolism and represent the most important component in anaerobic degradation of organic compounds (Whitman *et al.*, 1992). Methanogens live under extreme conditions of strict anaerobiosis in rice field soils, sludge sediments, swamped soils, plant xylem and the digestive tract of animals (Chin & Conrad, 1995). However, some species of methanogens can be relatively tolerant to oxygen exposure, surviving several hours in an oxygenated atmosphere during conditions of transient aerobiosis. *Methanosarcina* species can survive in oxygenated paddy soils, indicating that they are aerotolerant to some degree. Cells began to produce methane and to grow again as soon as anoxic conditions were re-established (Fetzer *et al.*, 1993). *Methanobrevibacter arboriphilus* and *Methanobacterium thermoautotrophicum* remain viable upon exposure to air for up to 30 h and cells of *Methanosarcina barkeri* survive even longer because of the formation of cell flocs (Kiener & Leisinger, 1983). Some *Methanogenium*, *Methanobacterium*

and *Methanosarcina* species were detected even in aerobic ecosystems such as surfaces of vegetables (Elstner, 1990) and desert soils (Peters & Conrad, 1995). Methanogens thus must have adaptive capacities to deal with the transient presence of oxygen and the concomitant oxidative stress. However, the mechanisms and regulation of oxidative stress responses in methanogenic archaea represent an almost completely unexplored area of research.

When strictly anaerobic micro-organisms come into contact with O₂, generally hydrogen peroxide (H₂O₂), superoxide radical (O₂^{•−}) and hydroxyl radical (OH[•]) are generated by the autoxidation of reduced iron–sulfur proteins and/or flavoproteins, catecholamines and quinones (Touati, 1997; Storz & Imlay, 1999). These reactive oxygen species are strong oxidants, which can destroy peptide bonds, and cause the depolymerization of nucleic acids and oxidation of polysaccharides and polyunsaturated fatty acids (Elstner, 1990; Fridovich, 1995) if not immediately removed by the main enzymes of antioxidative defence: superoxide dismutase and catalase. Anaerobic micro-organisms need defence systems to enable them to survive transient exposure to aerobic conditions or various pollutants in the environment.

Superoxide dismutase (SOD, EC 1.15.1.1) catalyses the disproportionation of 2 O₂^{•−} to O₂ and H₂O₂ and thus

Abbreviations: SOD, superoxide dismutase; PQ, paraquat (methyl viologen).

protects cells from free superoxide radicals, the products of univalent reduction of O₂. Generally, microbial species with high SOD activity have high or moderate aerotolerance in comparison to species with low or no SOD activity (Hewitt & Morris, 1975; Privalle & Gregory, 1979; Gregory & Dapper, 1980). Fe-containing SODs have been purified and characterized in the following methanogenic archaea: *Methanobacterium bryantii* (Kirby *et al.*, 1981), *Methanobacterium thermoautotrophicum* (Takao *et al.*, 1991), *Methanosarcina barkeri* (Brioukhanov *et al.*, 2000) and *Methanobrevibacter arboriphilus* (Brioukhanov *et al.*, 2006).

Catalase (EC 1.11.1.6) catalyses the conversion of 2 H₂O₂ to O₂ and H₂O, using H₂O₂ as the electron donor. Anaerobes that can endure only a short-term contact with O₂ do not need an obligatory catalase activity, unlike aerobic organisms, because H₂O₂ can be decomposed spontaneously or by non-enzymic mechanisms of defence (Hewitt & Morris, 1975; Fridovich, 1995). Nevertheless, catalase activity was found even in the cells of strictly anaerobic methanogenic archaea of the genus *Methanobrevibacter* (Leadbetter & Breznak, 1996). Haem-containing monofunctional catalases of *Methanosarcina barkeri* (Shima *et al.*, 1999) and *Methanobrevibacter arboriphilus* (Shima *et al.*, 2001) have been characterized, and the corresponding genes were cloned and sequenced.

Methanogenic archaea also contain additional enzymes of antioxidative defence as part of alternative oxidative stress response systems, such as the F₄₂₀:H₂ oxidase of *Methanobrevibacter arboriphilus*, involved in O₂ detoxification (Seedorf *et al.*, 2004), desulfoferrodoxin and rubrerythrin of *Methanobacterium thermoautotrophicum* (Smith *et al.*, 1997), and desulfoferrodoxin of *Methanococcus jannaschii* (Bult *et al.*, 1996); genes encoding these were found in the sequenced genomes.

In aerobic micro-organisms the physiological, biochemical and genetic responses to different oxidative stress conditions are complex and subtly regulated by two major transcriptional factors, OxyR and SoxRS, and intimately coupled to other regulatory networks in the cell; these responses have been quite well investigated to date (Lynch & Lin, 1996; Rosner & Storz, 1997; Storz & Imlay, 1999).

Significantly less is known about the molecular mechanisms and regulation of the oxidative stress responses that are required for the relative aerotolerance of strict anaerobic micro-organisms. Such responses have to be quite fast and fully induced, otherwise damage of the cell macromolecules by toxic oxygen derivatives will prevent the further expression of the antioxidative defence system. The oxidative stress responses in several species of strictly anaerobic bacteria have been studied in some detail. O₂ exposure leads to a strong increase of the specific activity of SOD in *Bacteroides thetaiotaomicron* (Pennington & Gregory, 1986). Aeration also induced the synthesis of SOD in the cells of *Porphyromonas gingivalis*, intensifying their virulence (Amano *et al.*, 1992; Lynch & Kuramitsu, 1999). On treatment of *Bacteroides*

fragilis with O₂, paraquat (PQ) or H₂O₂ an immediate *de novo* synthesis of more than 28 proteins starts, including catalase and SOD. The oxidative stresses induced by H₂O₂ or PQ are similar but not identical to the response induced by O₂ (Rocha & Smith, 1997; Rocha *et al.*, 2003). These regulated and adaptive responses suggest the involvement of transcriptional regulators, one of which has been identified as OxyR (Rocha *et al.*, 2003). An increase in the expression of the catalase of *B. fragilis* in response to aeration and to entry into the stationary growth phase is similar to adaptive response with involvement of hydroperoxidase II in *Escherichia coli* (Rocha & Smith, 1995, 1997; Rocha *et al.*, 1996). Recently the genes involved in the adaptive response to oxidative stress in *Clostridium perfringens* were identified. Among them were the genes encoding SOD, catalase, alkyl hydroperoxide reductase and ATP-dependent RNA helicase (Briolat & Reyssset, 2002; Jean *et al.*, 2004).

The regulation of antioxidative defence system in anaerobes and the number of genes involved in oxidative stress response remain unclear, especially in the strictly anaerobic archaea. To the best of our knowledge there are no data in the literature on the regulation of catalase and SOD activities at the cellular and molecular levels in methanogenic archaea in response to transient oxic/anoxic conditions. In a first step towards the characterization of the oxidative stress response, and the regulation of expression of the main enzymes of antioxidative defence, we purified and characterized the haem-containing monofunctional catalase (Shima *et al.*, 1999) and the iron SOD (Brioukhanov *et al.*, 2000) from *Methanosarcina barkeri*. Subsequently, their encoding genes (*kat* and *sod*) were cloned and sequenced (Shima *et al.*, 1999; Brioukhanov *et al.*, 2000). In the present article we report on the regulation of catalase and SOD expression in *M. barkeri* under different oxidative stresses, at both the mRNA and protein (enzyme activity) level.

METHODS

Strain and growth conditions. *Methanosarcina barkeri* strain Fusaro (DSMZ 804) was from the Deutsche Sammlung von Mikroorganismen und Zellkulturen. It was grown anaerobically (Hungate, 1967) on 1·2% (v/v) methanol at 37 °C in 250 ml bottles sealed with a butyl rubber stopper and an aluminium cap containing 100 ml medium as described previously (Karrasch *et al.*, 1989). For induction of peroxide stress and PQ stress responses the cultures were treated with freshly prepared and filter-sterilized anaerobic solutions of H₂O₂ and PQ (methyl viologen dichloride hydrate, Fluka Chemie). Growth was followed by measuring OD₅₅₀ at regular intervals (1 cm path-length cuvette, Jasco V-550 spectrophotometer).

Preparation of cell-free extracts. To measure specific activities of SOD and catalase, cell-free extracts of *M. barkeri* were made. Cells were harvested (9000 g, 4 °C, 20 min) in the late-exponential phase of growth and suspended in ice-cold 50 mM potassium phosphate buffer, pH 7·8. Cells were disrupted by ultrasonication, using a Branson Sonifier 450 (8 × 30 s with 2 min interim cooling in ice). Cell debris was removed by centrifugation for 20 min at 20 000 g and 4 °C, and the supernatant was stored at -20 °C.

SOD specific activity determination. SOD activity was determined spectrophotometrically at 25 °C (1 cm cuvette, Jasco V-550

spectrophotometer) by the xanthine oxidase–cytochrome *c* method (McCord & Fridovich, 1969). The 0.7 ml assay mixture contained: 50 mM potassium phosphate, pH 7.8; 0.1 mM EDTA; 50 µM xanthine (sodium salt, Serva); 1.7 mU xanthine oxidase (Serva); 10 µM cytochrome *c* (Merck). The reduction of cytochrome *c* by O_2^- , which was generated from O_2 by reduction with xanthine, was followed by measuring A_{550} . One unit (U) of SOD activity was defined as the amount of enzyme required to inhibit the reduction rate of cytochrome *c* by 50 % (McCord & Fridovich, 1969).

Catalase specific activity determination. Catalase activity was determined spectrophotometrically at 25 °C (1 cm cuvette, Jasco V-550 spectrophotometer) by monitoring the decrease in A_{240} ($\epsilon_{240} = 39.4 \text{ M}^{-1} \text{ cm}^{-1}$) of 13 mM H_2O_2 in 50 mM Tris/HCl buffer, pH 8.0 (Beers & Sizer, 1952; Nelson & Kiesow, 1972). One unit (U) of activity was defined as the amount of enzyme that catalyses the oxidation of 1 µmol $H_2O_2 \text{ min}^{-1}$ under the assay conditions.

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

Gene amplification. Probes for the *sod* and *kat* genes were obtained by PCR using as template genomic DNA from *M. barkeri*, isolated as described previously (Jarrell *et al.*, 1992). The nucleotide sequences of the *sod* and *kat* genes from *M. barkeri* strain Fusaro are available under accession numbers AJ272498 and AJ005939, respectively, in the GenBank/EMBL/DDBJ database. The oligonucleotide primers 5'-AAACCCGGGATGGCCAAAGAATTGTACAA-3' (forward for *sod*), 5'-AAACTCGAGTTATTTCCTCATTTTCCTGAA-3' (reverse for *sod*), 5'-AAACCCGGGATGGGTGAAAAGAATTCTTC-3' (forward for *kat*) and 5'-AAACTCGAGTTATCTCTCAGTTGCTCGG-3' (reverse for *kat*) were derived from the nucleotide sequences of the corresponding genes (Microsynth). The corresponding primers were located on the both ends of the coding sequence of the whole gene. The 25 µl PCR mixture contained 2.5 ng genomic DNA of *M. barkeri* strain Fusaro, 1.3 U *Taq* DNA polymerase (Stratagene), 250 µM deoxynucleoside triphosphates, 1.5 mM $MgCl_2$ and 0.5 µM concentrations of each of the two primers. The temperature programme was 5 min at 95 °C, 30 cycles of 30 s at 94 °C, 1 min at 55 °C and 1 min at 72 °C, and a final step of 5 min at 72 °C. The PCR products were isolated from the agarose gel using the JetSorb Gel Extraction kit (Genomed) and suspended in 20 µl TE buffer (10 mM Tris/HCl, 1 mM EDTA, pH 8.0). Probes for the *sod* and *kat* genes were cloned into pGEM-T Easy vector (Promega Catalys), using *E. coli* DH5 α competent cells, and re-isolated by digestion with *Sma*I and *Xho*I restriction enzymes (Stratagene).

RNA isolation and dot-blot analysis. Total RNA was isolated using the following procedure. Cultures were grown to late-exponential phase ($OD_{550} 1.5$) and 2 ml aliquots were immediately centrifuged at 10 000 g for 10 min at 4 °C. After centrifugation, the cell pellet was suspended in 450 µl sterile AE buffer (30 mM sodium acetate pH 5.5, 10 mM Na_2EDTA and 1.5 %, w/v, SDS). Then immediate extraction with 450 µl TRIzol reagent (Life Technologies) was carried out at 65 °C for 5 min followed by centrifugation at 10 000 g for 10 min. The aqueous phase was precipitated with 250 µl cold 96 % ethanol. Then the RNeasy Mini kit (Qiagen) was used for the final isolation of RNA (steps 5–10 according to the RNeasy Mini protocol for isolation of total RNA from bacteria). The RNA pellet was dissolved in RNase-free water and stored at –70 °C. RNA samples with RNA loading dye (50 %, w/v, sucrose solution in 30 %, v/v, glycerol, 15 mg bromphenol blue ml^{-1}) were checked by electrophoresis (5–6 V cm^{-1} , 20 °C) in 1 % (w/v) agarose gel containing 1× TAE buffer (40 mM Tris/acetate pH 8.3, 1 mM EDTA). The concentration of total RNA was determined by measuring A_{260} .

RNA samples (10 µg) from the cultures exposed to different oxidative stress conditions were transferred to a nylon membrane (Qiagen) by capillary action in $10 \times SSC$ ($1 \times SSC$ is 0.15 M NaCl plus 0.015 M sodium citrate) and cross-linked by UV irradiation (Sambrook *et al.*, 1989). The probes (10 ng) used for dot-blot analysis were a 612 bp *Sma*I–*Xho*I DNA fragment of the *sod* gene and a 1518 bp *Sma*I–*Xho*I DNA fragment of the *kat* gene. Probes were denatured by boiling in a water bath for 10 min and labelled with DIG (Digoxigenin-11-dUTP) by random primer reaction with a DIG High Prime DNA labelling and detection starter kit (Roche Diagnostics). Hybridization, immunological detection of the hybridized probes with anti-digoxigenin-AP Fab fragments and then visualization with the chemiluminescence substrate CSPD were performed following the instruction manual from the kit. Autoradiographs were obtained by exposing the membranes to X-ray film (Eastman Kodak) for 30 min at 20 °C in the dark.

Chemicals. Chemicals were from Fluka Chemie and of analytical grade.

Statistical analysis. Initial growth inhibition experiments were replicated three times, while the stress treatments involving the enzyme activity measurements were replicated five times. The data were analysed by the SigmaPlot and SigmaStat programs (Systat Software). Dot-blot hybridization experiments were replicated twice. The densitometric quantification of the signals after dot-blot hybridization was done with the help of the ImageJ program (Wayne Rasband).

RESULTS

Determination of growth-inhibitory concentrations of PQ and H_2O_2

To investigate the influence of PQ as the source of superoxide radicals on the growth of *Methanosarcina barkeri* and determine the appropriate sublethal doses of PQ to be used for subsequent experiments, the following experiment was carried out. *M. barkeri* cultures were grown under standard conditions for 24 h, after which various amounts of PQ solution were added to the culture. Growth was not influenced by 25 µM PQ; between 50 and 100 µM PQ growth was retarded and at 200 µM PQ it was completely inhibited (Fig. 1a).

The same experimental setup was used to determine critical H_2O_2 concentrations. A concentration of 100 µM H_2O_2 did not affect the growth of *M. barkeri*, but 0.2–0.8 mM H_2O_2 strongly inhibited its growth. H_2O_2 at 1.6 mM completely prevented growth (Fig. 1b).

Based on these results, subsequent exposure experiments were performed with 0.4 mM and 1.3 mM H_2O_2 , and with 50 µM and 160 µM PQ, representing effective but non-lethal concentrations.

Effect of exposure to PQ and H_2O_2 on the specific activities of SOD and catalase

The specific activities of the enzymes of antioxidative defence in cell-free extracts of *M. barkeri* after treatment of cultures with PQ and H_2O_2 were measured as an initial investigation of the oxidative stress response. PQ was added to the culture medium at concentrations of 50 µM (25 %

Fig. 1. Effect of PQ (a) and H₂O₂ (b) on growth of *M. barkeri*. The data plotted are means $\pm$ SD from three independent experiments.

growth inhibition concentration) and 160 μ M (80 % growth inhibition concentration) for 30 min, 2 h, 4 h and 8 h before the cultures were harvested for measurements. H₂O₂ stress was induced by adding 0.4 mM H₂O₂ (25 % growth inhibition concentration) and 1.3 mM H₂O₂ (80 % growth inhibition concentration), also for 30 min, 2 h, 4 h and 8 h before activity measurements.

Upon addition of PQ (final concentration 160 μ M), the specific activity of SOD increased nearly twofold after 2 h exposure, and was about 1.5-fold higher than the control after 4 h exposure (Fig. 2a). The lower concentration (50 μ M) of PQ had an insignificant effect on specific activity of SOD (0.5–8 h exposure). The specific activity of SOD increased already after 0.5 h exposure of cells to H₂O₂ at a final concentration of 1.3 mM and reached its maximum (nearly twofold increase) after 2 h exposure (Fig. 2b).

The specific activity of catalase increased nearly twofold after 2 h exposure to PQ at a final concentration of 160 μ M (Fig. 3a). PQ at the lower concentration of 50 μ M had only little effect on catalase activity after 4 h exposure. H₂O₂ (final concentration 1.3 mM) increased the specific activity of catalase 2.5-fold after 2 h exposure and then the activity decreased (Fig. 3b). Almost no effect on specific activity of

Fig. 2. Activity of SOD in cell-free extracts of *M. barkeri* after addition of PQ (a) or H₂O₂ (b) to the culture. The data plotted are means $\pm$ SD from five independent experiments.

catalase was observed after addition of H₂O₂ at the lower concentration of 0.4 mM after 0.5–8 h exposure.

Effect of exposure to PQ and H₂O₂ on the expression of *sod* and *kat* genes

To study whether the increased SOD and catalase activities were the result of transcriptional up-regulation of the corresponding *sod* and *kat* genes of *M. barkeri*, dot-blot analysis was done, using identical oxidative stress conditions to those described above.

PQ at a final concentration of 160 μ M significantly induced the synthesis of *sod* mRNA. The maximum (fivefold) up-regulation of the *sod* gene was observed already after 0.5 h and 2 h exposure to PQ (Fig. 4a), which corresponds to the increase of specific activity of SOD (Fig. 2a). H₂O₂ at a final concentration of 1.3 mM also influenced the synthesis of *sod* mRNA after 2 h exposure (fourfold up-regulation), which resembled the increase in SOD specific activity (Fig. 2b). The addition of lower concentrations of the two oxidizing agents did not strongly induce the expression of the *sod* gene.

The levels of *kat* mRNA increased somewhat following 2 h and 4 h exposure of cells to PQ at a final concentration of

Fig. 3. Activity of catalase in cell-free extracts of *M. barkeri* after addition of PQ (a) or H₂O₂ (b) to the culture. The data plotted are means $\pm$ SD from five independent experiments.

160 μ M, and increased to a greater extent after 2 h (almost tenfold) and 4 h (more than fivefold) exposure to H₂O₂ at a final concentration of 1.3 mM (Fig. 4b). Lower concentrations of PQ (50 μ M) and H₂O₂ (0.4 mM) did not have a significant effect on *kat* gene expression.

DISCUSSION

Methanosarcina barkeri Fusaro is a very strictly anaerobic methanogen which needs a redox potential of less than -0.3 V for growth (Hungate, 1967). *Methanosarcina* species can, however, survive transient aerobiosis and remains viable in the presence of O₂ for at least 48 h through the formation of cell flocs (Zhilina, 1972; Kiener & Leisinger, 1983). We have shown here that *M. barkeri* can grow after addition of 0.8 mM H₂O₂ or of 100 μ M PQ to the culture medium. *M. barkeri* has a SOD activity which is two times lower than the specific SOD activity in *Methanobrevibacter arboriphilus* (Brioukhanov & Netrusov, 2004), and *M. barkeri* is nearly five times more sensitive to PQ exposure compared to *M. arboriphilus* (Brioukhanov *et al.*, 2006). This confirms the relationship between SOD activity in the cells and aerotolerance of the micro-organism.

Fig. 4. Dot-blot hybridization analysis of RNA from *M. barkeri* (late-exponential phase of growth) after exposure to PQ or H₂O₂. Dot-blot were probed with *sod* (a) and *kat* (b) genes. Numbers below the spots represent the integrated density as compared to the reference probes (*sod* and *kat* from anaerobic cultures: bottom panels).

It should be noted that the mechanism of PQ toxicity in anaerobic conditions still remains unclear. One explanation is that culture medium and PQ solution even after standard preparation according to the Hungate technique could contain traces of oxygen, enough to give rise to superoxide. Alternative explanations are that under anaerobic conditions PQ can cause the oxidation of formate, deoxyribose, etc. (Winterbourn & Sutton, 1984), or can react as a strong redox-cycling agent with different electron acceptors, traces of H₂O₂ or transition metals (Weidauer *et al.*, 2002), which leads to accumulation of reactive oxygen species under anaerobiosis.

The specific activity of catalase in cell-free extracts of *M. barkeri* increased 2.5-fold after 2 h exposure to H₂O₂ (final concentration of 1.3 mM); this does not represent a very fast response, in comparison with the 6–8 h doubling time of *M. barkeri*. Lower concentration of H₂O₂ (0.4 mM) had almost no effect on the activities of the enzymes of anti-oxidative defence, indicating that small amounts of H₂O₂ are probably decomposed by constitutive catalase, by

non-enzymic mechanisms of antioxidative defence or spontaneously. Generally, it should be noted that the increase of specific activities even under the most severe tested stress conditions (160 μ M PQ, 1.3 mM H_2O_2) was not high (two-fold for SOD and three-fold for catalase) and the maximal activities decreased after 2–4 h exposure to PQ or H_2O_2 .

It was shown earlier (Briukhanov *et al.*, 2002) that the specific activity of SOD reached its maximum (twofold above the activity in the mid-exponential phase of growth) in the stationary phase during cultivation of *M. barkeri* on methanol under anaerobic conditions. Catalase activity practically did not change during the anaerobic growth on methanol (Briukhanov *et al.*, 2002). Probably, the cells need to intensify the antioxidative defence not only under unfavourable oxic conditions, but also when cultures are approaching the stationary phase of growth (conditions of cell starvation with dormant metabolism), where the rate of cell death and the possibility of formation of superoxide radicals are high (Fridovich, 1995).

Similar effects of oxidative stresses on SOD and catalase have been described for other strict anaerobes. Aeration increased the specific activity of SOD and the level of corresponding mRNA two- and threefold in *P. gingivalis* (Amano *et al.*, 1992; Lynch & Kuramitsu, 1999) and the SOD activity in *B. thetaiotaomicron* cells fourfold (Pennington & Gregory, 1986). Oxidative stress induced a tenfold increase in SOD activity (Abdollahi & Wimpenny, 1990) and catalase activity (Fareleira *et al.*, 2003) in the sulfate-reducing bacterium *Desulfovibrio desulfuricans*. The increase of SOD activity, accompanied by the *de novo* synthesis of SOD, continued for 90 min after oxygen treatment of *B. fragilis* cells (Priville & Gregory, 1979). Our data show that exposure of *M. barkeri* to H_2O_2 and PQ also causes *de novo* SOD synthesis (Fig. 4a). The level of *katB* mRNA increased more than 15-fold upon exposure to O_2 , PQ or H_2O_2 in mid-exponential-phase cultures of *B. fragilis* (Rocha & Smith, 1997). In *M. barkeri* cells from the late-exponential phase of growth the level of *kat* mRNA increased almost 10-fold upon exposure to H_2O_2 (Fig. 4b).

The higher concentrations of PQ (160 μ M) and H_2O_2 (1.3 mM) tested had statistically significant effects on the specific activities of SOD and catalase of *M. barkeri*. The same concentrations also induced the corresponding *sod* and *kat* genes. PQ (160 μ M) and H_2O_2 (1.3 mM) showed similar effects on SOD specific activity. However, the specific activity of catalase was 1.5 times higher and the expression of the *kat* gene of *M. barkeri* was five times higher after exposure to 1.3 mM H_2O_2 , which is the direct substrate for catalase, compared to 160 μ M PQ in the culture medium. In the latter case the intracellular superoxide, produced by PQ, must be previously converted to H_2O_2 . The PQ treatment had a more significant effect on catalase activity, as would be expected. The regulation of antioxidative defence system in the cells of *M. barkeri* may be more complex and PQ may strongly induce all components of the system, including

catalase. It was also obvious that sublethal concentrations of PQ had a less strong effect on *kat* gene expression in comparison with sublethal concentrations of H_2O_2 (maximally 1.8-fold compared to 9.5-fold with H_2O_2), and PQ had a strong stimulating effect on transcriptional up-regulation of the *sod* gene (160 μ M, 0.5 h exposure; Fig. 4a).

Here we have shown at the enzyme activity and transcriptional levels of SOD and catalase, the main enzymes of antioxidative defence, are up-regulated and induced in the cells of a strictly anaerobic methanogenic archaeon. These enzymes possibly play a significant role in the protection of *M. barkeri* against the toxic effects of reactive oxygen species ($\text{O}_2^{\cdot-}$ and H_2O_2), providing the relatively high aerotolerance of this methanogen.

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