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Isolates of *Comamonas* spp. exhibiting catalase and peroxidase activities and diversity of their responses to oxidative stress

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ABSTRACT

For survival isolates of *Comamonas testosteroni* CCM 1931, *C. testosteroni* K3, *C. terrigena* N3H or N1C and *C. terrigena* CCM 2409, selected largely from polluted environments, the production of catalase and dianisidine-peroxidase activity was important. Electrophoretic resolution of cell-free extracts of aerobically grown strains in Luria-Bertani medium during the exponential phase revealed distinctive expression of catalatic and peroxidatic activities detected with 3,3′-diaminobenzidine tetrahydrochloride (DAB). The protection of isolates from 20 or 40 mM H_2O_2 stress was characterized with a considerable diversity in catalase and peroxidase responses that resulted from hydroperoxidase's variant of original isolates, indicating also a selective pressure of environment. Results indicate catalase to be important for adaptation of cultures to high concentration of 60 mM H_2O_2. The greatest appreciable differences in sensitivity to toxic effect of H_2O_2 (20 or 40 mM) treatment between individual isolates and their adapted variants during the growth were observed until the middle of exponential phase. Isolates exhibited diversity in catalases responses to possible contaminants *o*-or *p*-phenylenediamine (PDA) as well. Only positional isomer *p*-PDA (1 or 2 mM) stimulated catalase activity unlike from isomer *o*-PDA in *C. terrigena* N3H cells. The study can contribute to understanding of bacterial antioxidative enzymatic responses in the presence of possible physiological stress resulting mainly from environmental pollutants.

1. Introduction

Catalases are involved as one of the mechanisms used to protect cells against the damage caused by reactive oxygen species (ROS) to cellular components, including nucleic acids, lipids and proteins (Imlay and Linn, 1998). They decompose, very efficiently, substances containing the peroxidic bond, preferentially, hydrogen peroxide, to oxygen and water, thus forming an essential part of the defense mechanism against oxidative stress (Engelmann and Hecker, 1996). Typical catalases reveal only negligible peroxidatic activity. Bifunctional enzymes combining both catalatic and peroxidatic activities known as catalase–peroxidase are found in prokaryotes and marginally in some fungi and protists (Zámocký et al., 2008). Lower catalase and higher peroxidase activity with large aromatic 1-electron substrates is characteristic for this enzyme.

Challenge by reactive oxygen species occurs from normal metabolism of microorganisms as well as from external sources. Many pollutants in the soils are subjected to oxidation processes resulting in the formation of reactive intermediates that react with oxygen producing the reactive oxygen species. Survival of microorganisms as a response to environmental oxidative stress is accompanied with the accurate regulation of catalase and peroxidase activities, including a possible alteration in the expression of isozymes (Hishinuma et al., 2006).

Soil bacteria of the family Comamonadaceae are known for their broad catabolic diversity in the degradation of various xenobiotics. Isolate *C. terrigena* N3H from soil contaminated with crude oil products exhibited much higher total catalase activity than the same species from unpolluted soil. Electrophoretic resolution of isolate cell-free extract revealed bands of catalase-1 and catalase-2, whereas in the control, only catalase-1 was detected (Godočíková et al., 2005). Isolates of *Comamonas* selected from soil polluted with crude oil products and similarly, isolates of *Aspergillus niger* isolated from mine surroundings contaminated with heavy metals exhibited higher catalases activities and were more tolerant to the exogenic toxic stress than the same cultures from control area (Bučková et al., 2005; Godočíková et al., 2005).

Obviously, catalases are not only essential for the protection against oxidative stress, but with peroxidation they can also participate in the metabolic changes, for example of some phenolic compounds, ubiquitous contaminants in soil and water. *C. terrigena* N3H lacks the key enzyme for degradation of phenols, phenolhydroxylase. Phenol peroxidation, the formation of the

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secondary product 2,2′-biphenyl, followed fluorimetrically was attributed to the peroxidatic action of a catalases (Zámocký et al., 2001). *Mycobacterium* sp. strain PYR-1 degrades polycyclic aromatic hydrocarbons, environmental pollutants. It was shown, that inducible catalase–peroxidase of *katG* gene, of this culture is involved in molecular mechanisms of degradation of these pollutants (Wang et al., 2000).

In this report, bacterial *Comamonas* isolates, from environments with long-term pollution and which exhibited distinct catalase and *o*-dianisidine-peroxidase activities were screened and identified. The diversity of catalases responses to exogenic oxidative stress under laboratory conditions was followed during the exponential phase of growth when cells are able to break down the pollutants (Godočíková et al., 2004). Enzymes that participate in the protection of cells against oxidative stress damage are also involved in the metabolic oxidation of large aromatic substrates; consequently our results can contribute to a better understanding of the physiological role of catalases in bacterial responses to polluted environments.

2. Materials and methods

2.1. Chemicals and strains

Chemicals, including tryptone, hydrogen peroxide, potassium ferricyanide, ferric chloride and 3, 3-diaminobenzidine tetrahydrochloride (DAB) were purchased from Sigma Chemical Co. (USA), *o*-or *p*-dianisidine dihydrochloride from LLC (Germany), *o*- or *p*-phenylenediamine, Biomedicals, USA hydrogen peroxide p.a., Slavus, Slovakia and meat extract-peptone agar from Imuna (Slovakia). Chemicals for polyacrylamide gel electrophoresis were obtained from BioRad (USA). *C. testosteroni* K3 isolate was selected from sludge of wastewater containing high amount of heavy metals. The *C. terrigena* N3H and *C. terrigena* N1C isolates were obtained from soils long-time contaminated with crude oil products (Prokšová et al., 1996). *C. testosteroni* CCM 1931 (ATCC 11996) and *C. terrigena* CCM 2409 (ATCC 8461) were provided from the Czech Collection of Microorganisms, Masaryk University, Brno, Czech Republic. The strains (isolates and cultures from the collections) were maintained on nutrient agar slants at 4 °C and subcultured every 2 months.

2.2. Growth conditions and stress treatments

Bacterial cultures were grown in Luria-Bertani medium (10 g tryptone, 5 g yeast extract and 5 g NaCl) at 30 °C. Overnight culture (0.5 mL) in Luria-Bertani medium was transferred to a flask containing 50 mL of the growth medium and shaken at 200 rpm at 30 °C. The growth of all strains was monitored by the absorbance at 610 nm. When growth reached the one fourth of the beginning (BE), or the middle of exponential phase (ME), or the entry to stationary phase (ES), ~~the cells were exposed to an added stress agent for 1 h.~~ The kinetics of catalases induction was revealed by labeling at these times. Then they were harvested by centrifugation at 5000*g* for 10 min at 4 °C, and washed. ~~The stress conditions were achieved by exposing the cells to one of the following oxidative agents: 20 or 40 mM hydrogen peroxide (H_2O_2), 1 or 2 mM *o*- or *p*- phenylenediamine (*o*-PDA and *p*-PDA).~~ Oxidative challenge involved adding oxidative stressor to a culture aliquot (ca. 2×10^8 cells). Viable cell counts were determined by serial dilution of the samples and dropping on nutrient agar plates.

For determining microbial counts the most probable number (MPN) procedure was used and the experimental design used in this experiment was that described (Pepper et al., 1995; Alexander, 1982). The actual concentrations of phenylenediamines (PDAs) were confirmed using the method described by Norwitz and Keliher (1982). H_2O_2 concentrations were determined by manganometry, which uses the quantitative reduction of $KMnO_4$ to $MnSO_4$ using H_2O_2 in the presence of H_2SO_4.

2.3. Stepwise H_2O_2-adapted cultures

The nutrient medium was inoculated with bacterial isolates (*C. testosteroni* K3, *C. testosteroni* CCM 1931, *C. terrigena* N3H, *C. terrigena* N1C, *C. terrigena* CCM 2409) and cultivated at 30 °C. When growth was visible, samples were subcultivated (2% v/v) into identical fresh media containing H_2O_2. After performing a number of subcultivations with gradually increased concentrations of the oxidative stressor, samples (0.1 ml) were plated on nutrient agar and incubated at 30 °C for 48 h. Individual colonies were selected for further growth in liquid or solid medium. The final concentration of H_2O_2 in the cultivation medium was 60 mM.

2.4. Enzyme activity determination

Mechanical disruption of frozen cells was performed in 50 mM sodium phosphate, 2 mM EDTA (pH 8.2) at 4 °C with glass beads (diameter 0.4 μm). The homogenates were centrifuged at 15 000*g* for 10 min. Supernatants were used for the determination of enzymatic activities. Catalase was determined at pH 7.0 by monitoring the decomposition of H_2O_2 at 240 nm with an extinction coefficient of 43.6 M^{-1} cm^{-1}. One unit of catalase activity (U) was defined as the amount of enzyme that catalyzes the decomposition of 1 μmol of H_2O_2 per minute (Roggenkamp et al., 1974). Peroxidase was assayed at pH 5.5 by monitoring the oxidation of *o*-dianisidine dihydrochloride at 460 nm with an extinction coefficient of 11.3×10^3 M^{-1} cm^{-1}. One unit of peroxide activity (U) was defined as the amount of activity that produces 1 μmol of oxidized *o*-dianisidine per minute (Claiborne and Fridovich, 1979). Total protein was quantitated using a Coomassie stain-based assay (Pierce, Rockford, IL).

2.5. Gel electrophoresis and staining

Native polyacrylamide gels with a linear gradient (4–18%) were stained for either catalase or peroxidase activity. Catalase activity was visualized after a brief incubation in 0.003% hydrogen peroxide and followed by a freshly prepared mixture of 2% potassium ferricyanide and 2% ferric chloride (Woodbury et al., 1971). Peroxidase activity was visualized with 3,3′-diaminobenzidine tetrahydrochloride (DAB) using the method described by Wayne and Diaz (1986).

2.6. Statistical analysis

All the experiments in this study were performed in triplicates and analyzed individually. An average standard deviation was calculated. The bars shown in the figures indicate a standard error of the mean ($n=3$).

3. Results

3.1. Growth and production of catalases

The growth of isolates under standard cultivation conditions in LB medium reached stationary phase in 12 h. Strains *C. testosteroni* K3, *C. terrigena* N3H and *C. terrigena* N1C exhibited a gradual increase in catalase activity during aerobic growth, while strains *C. testosteroni* CCM 1931 and *C. terrigena* CCM 2409 showed only a mild rise in dianisidine-peroxidase activity and relatively lower levels of catalase activity (Fig. 1).

Electrophoretic resolution of cell-free extracts of individual isolates grown in exponential phase revealed differences in the expression of catalase and peroxidase activities, which are most likely due to selective environmental pressure. *C. testosteroni* K3 expressed double band showing catalase activity (CAT1), but did not exhibit peroxidase activity by 3,3′-diaminobenzidine tetrahydrochloride (DAB) staining. *C. testosteroni* 1931 expressed a catalase band which corresponded to CAT1, but which also showed peroxidatic activity (Fig. 2). A *C. terrigena* N3H cell-free extract expressed two catalase bands (CAT1 and CAT2) and *C. terrigena* N1C expressed only one catalase band (CAT1); DAB staining did not show peroxidase activity in the bands from these cultures, however (Fig. 3). *C. terrigena* CCM 2409 exhibited both catalase activity proportional to the size of CAT1, and peroxidase activity in CAT1 and CAT2 bands. The CAT2 band did not exhibit catalase activity, however, and appears to be only a peroxidase.

3.2. Survival and catalase responses of cultures to exogenic H_2O_2

We compared the sensitivity of individual isolates to exogenous hydrogen peroxide stress. Cultures in different phases of growth were stressed by toxic doses of H_2O_2 for 1 h and their survival was then measured by the counting of viable cells. The resistance of isolates to the exogenic effect of H_2O_2 (20 or 40 mM) during growth gradually increased (Table 1).

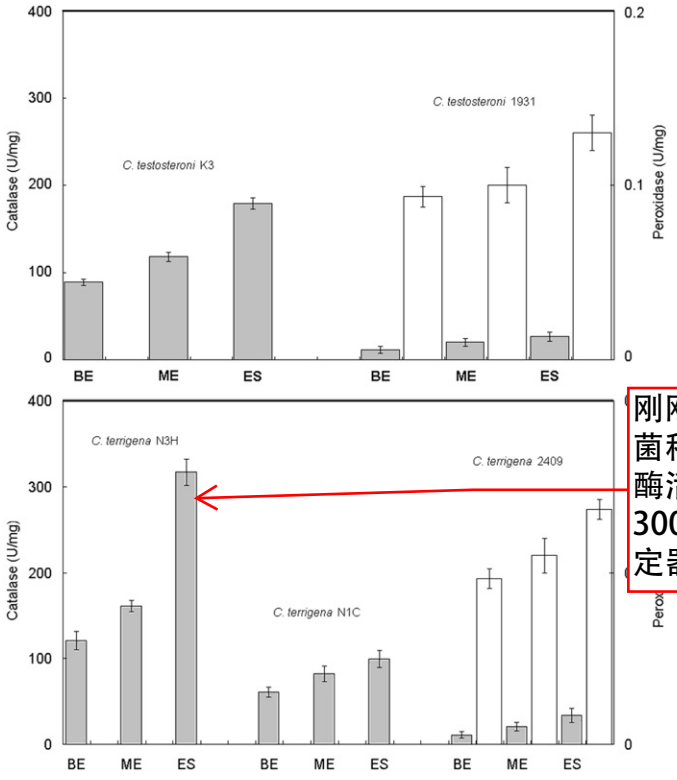

Fig. 1. Catalase (shaded columns) and peroxidase (unshaded) activities in crude extracts of *Comamonas* isolates grown in Luria-Bertani medium. Activities were measured at the beginning (BE) and middle (ME) of the exponential phase and at the entry into the stationary phase (ES) of growth.

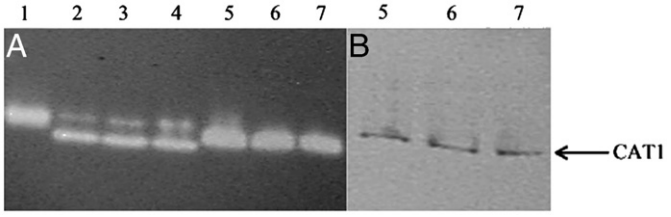

Fig. 2. Catalase (A) and peroxidase activities (B) in crude extracts of *Comamonas* isolates indicated by native polyacrylamide gel electrophoresis (4–18%). (A) Lanes: 1—a typical catalase from bovine liver (232 kDA); 2–4—*C. testosteroni* K3; 5–7—*C. testosteroni* CCM 1931. (B) Lanes 5–7—Peroxidase activity of *C. testosteroni* CCM 1931 was detected with 3,3′-diaminobenzidine tetrahydrochloride (DAB). Extracts were prepared from the beginning (BE) and middle (ME) of the exponential phase and from the entry into stationary phase (ES).

The defense of isolates to exogenic oxidative stress is characterized by a considerable diversity in catalase responses. They are due to a hydroperoxidase variant of the original isolate, indicating that the environment is exerting selective pressure. The effect of 20 or 40 mM H_2O_2 during the middle of exponential phase stimulated catalase activity in *C. testosteroni* K3, mildly stimulated it in *C. terrigena* N3H and N1C cultures, and visibly increased the peroxidase activity in the *C. testosteroni* CCM 1931 and *C. terrigena* CCM 2409 strains (Fig. 4).

To obtain variants of the chosen *Comamonas* spp. strains more resistant to H_2O_2 stress, individual isolates were subcultivated in gradually increasing (up to a maximum of 60 mM) concentrations of H_2O_2. Their higher resistance in comparison to the original isolates was confirmed by an inhibition zone test. The addition of 10 µl of 0.3 and 0.6 M H_2O_2 created empty zones around the original isolates, but not around the adapted cultures. A representative test for *C. testosteroni* CCM 1931 cultures is shown

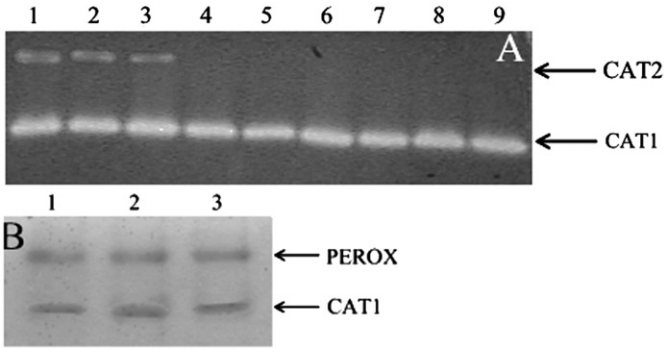

Fig. 3. Catalase (A) and peroxidase (B) activity in crude extracts of *C. terrigena* polyacrylamide gel electrophoresis (4–18%). (A) Lanes: 1–3—6—*C. terrigena* N1C; 7–9—*C. terrigena* CCM 2409. (B) Lanes: 1–M 2409. Extracts were prepared from the beginning (BE) and exponential phase and at the entry into stationary phase (ES).

growing isolates to exogenous H_2O_2 stress. BE—beginning of exponential phase, ME—middle of exponential phase and ES—entry into stationary phase.

Culture	Phase of growth	Survival after H_2O_2 treatment[a]	
		20 mM	40 mM
C. testosteroni 1931 isolate	BE	10 ± 2	9 ± 2
	ME	25 ± 2	15 ± 3
	ES	60 ± 4	36 ± 4
C. testosteroni 1931 H_2O_2 adapted	BE	31 ± 4	28 ± 4
	ME	40 ± 4	32 ± 4
	ES	79 ± 4	72 ± 3
C. testosteroni K3 isolate	BE	15 ± 4	10 ± 2
	ME	76 ± 3	60 ± 4
	ES	80 ± 4	76 ± 5
C. testosteroni K3 H_2O_2 adapted	BE	20 ± 2	18 ± 3
	ME	79 ± 5	65 ± 4
	ES	81 ± 5	79 ± 2
C. terrigena 2409 isolate	BE	10 ± 2	8 ± 1
	ME	43 ± 3	10 ± 2
	ES	80 ± 4	75 ± 4
C. terrigena 2409 H_2O_2 adapted	BE	21 ± 4	19 ± 2
	ME	55 ± 4	52 ± 3
	ES	82 ± 4	78 ± 4
C. terrigena N1C isolate	BE	55 ± 3	40 ± 4
	ME	63 ± 3	51 ± 3
	ES	66 ± 4	61 ± 3
C. terrigena N1C H_2O_2 adapted	BE	60 ± 3	52 ± 3
	ME	68 ± 3	60 ± 4
	ES	88 ± 4	81 ± 4
C. terrigena N3H isolate	BE	58 ± 3	40 ± 3
	ME	71 ± 3	55 ± 4
	ES	88 ± 5	80 ± 4
C. terrigena N3H H_2O_2 adapted	BE	65 ± 4	56 ± 3
	ME	95 ± 4	90 ± 4
	ES	91 ± 4	88 ± 4

Given are mean values ± SD for three independent measurements.

[a] % of survival compared to control cultures that were grown without oxidative stress.

in Fig. 5. The H_2O_2-adapted cultures exhibited a significant increase in catalase activity in all cultures during growth with a decrease in or absence of peroxidase activity (Table 2). Electrophoretic resolution of *C. terrigena* N3H adapted to 60 mM H_2O_2 revealed that increase in total catalase activity was mainly due to an increase in the expression of the CAT1 band to the detriment of the CAT2 band (Fig. 6).

Significant differences were observed during growth between the sensitivities of the original isolates and their adapted variants to treatment with 20 and 40 mM concentrations of H_2O_2 until the

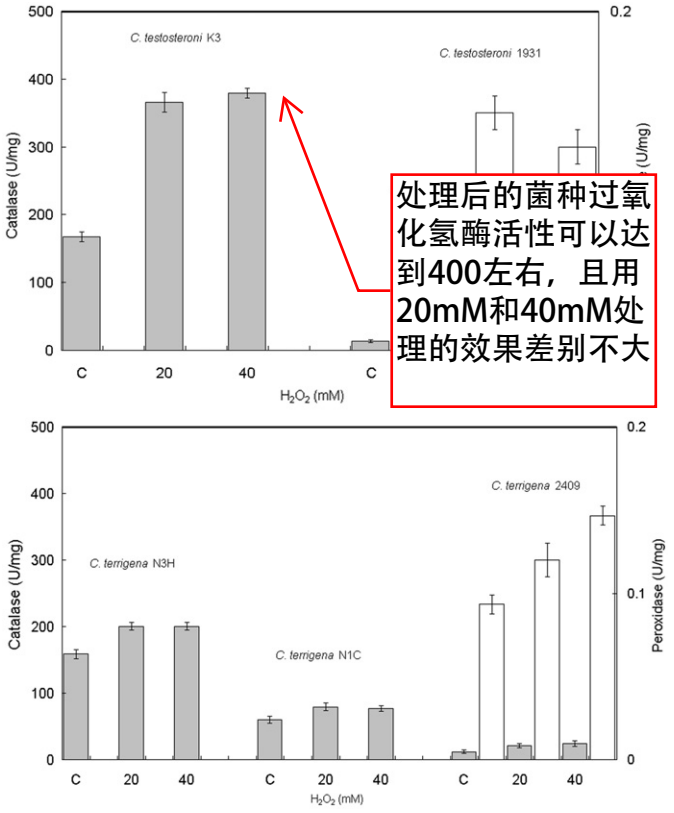

Fig. 4. Effect of H_2O_2 stress on the catalase (shaded) and peroxidase (unshaded) activities of *Comamonas* cultures at the middle of the exponential growth phase. Control (C)—activity in unstressed cultures.

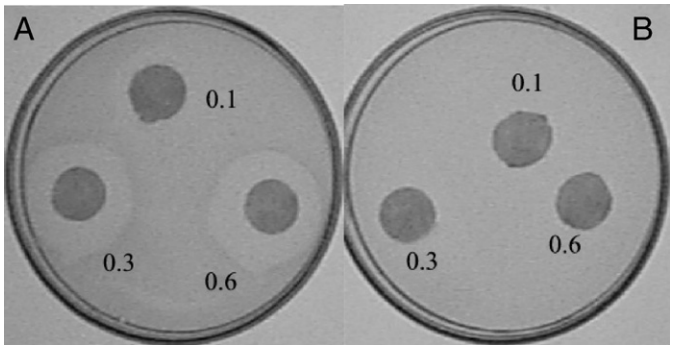

Fig. 5. Inhibition zone test for *C. testosteroni* CCM 1931. Cells of the original isolate (A) and its H_2O_2-adapted subculture (B) were grown overnight. The sterile filter paper disks contained 10 μl of 0.1 M, 0.3 M or 0.6 M H_2O_2.

Table 2
Relative changes in catalase and peroxidase activities in H_2O_2-adapted cultures in comparison to original isolates in the middle of the exponential growth phase.

Culture	Catalase activity (%)	Peroxidase activity (%)
C. testosteroni K3 H_2O_2 adapted	200 ± 10	0
C. testosteroni 1931 H_2O_2 adapted	540 ± 15	40 ± 2
C. terrigena N3H H_2O_2 adapted	390 ± 12	0
C. terrigena N1C H_2O_2 adapted	380 ± 10	0
C. terrigena 2409 H_2O_2 adapted	400 ± 13	60 ± 3

Given are mean values ± SD for three independent measurements.

middle of the exponential phase. The survival of the adapted cultures of *C. testosteroni* CCM 1931 and *C. terrigena* N3H in comparison to original isolates after 40 mM treatment was higher approximately 2.1 and 1.6 times, respectively. By the time the cultures began to enter stationary phase growth, the differences in the survival of adapted *Comamonas* spp. isolates in comparison to the original isolates were diminished or were negligible in *C. testosteroni* K3 and in *C. terrigena* N3H (Table 1).

3.3. Response of catalases to o- or p-phenylenediamine

Isolates also exhibited a diversity of catalase responses to the possible environmental contaminants *o*- and *p*-phenylenediamine (PDA). *o*-Phenylenediamine (1 and 2 mM concentrations) visibly stimulated peroxidase activity in *C. testosteroni* CCM 1931 and in *C. terrigena* CCM 2409, but only slightly in *C. terrigena* N3H and N1C strains, which exhibit no measurable peroxidase activity at

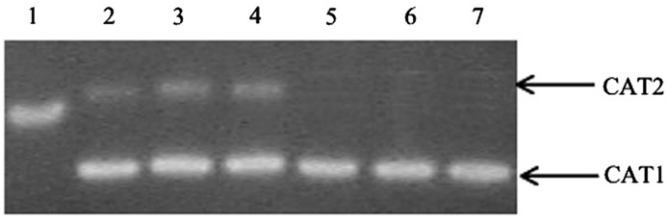

Fig. 6. Electrophoretic resolution of *C. terrigena* N3H catalases (PAGE 4–18%). Lanes: 1—a typical catalase from bovine liver (232 kDA); 2–4—isolate and 5–7—the strain adapted to 60 mM H_2O_2. Extracts were prepared from the beginning (BE) and middle (ME) of the exponential phase and from the entry into stationary phase (ES).

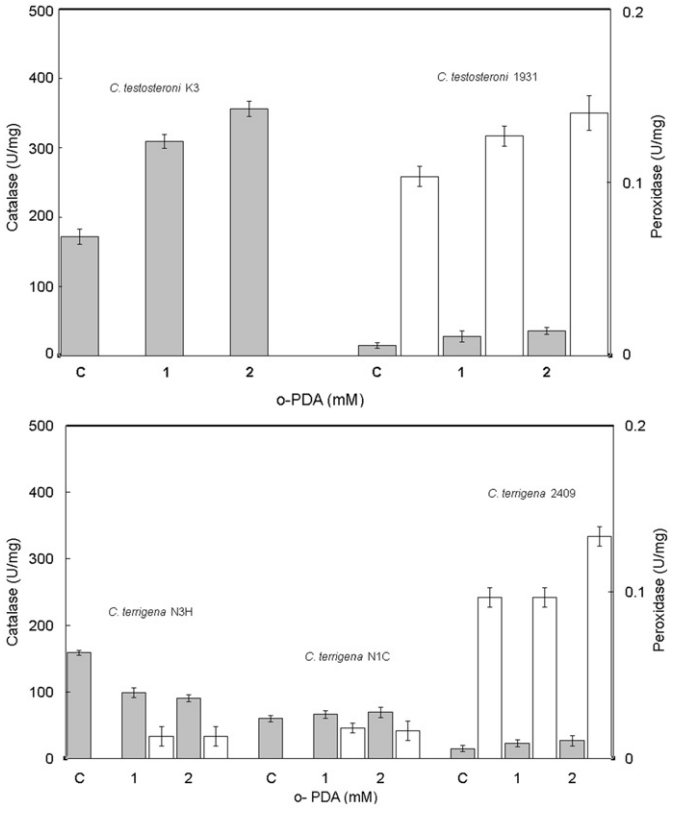

Fig. 7. The effect of *o*-phenylenediamine on the catalase (shaded) and peroxidase (unshaded) activities of *Comamonas* spp. at the middle of exponential phase. Control (C)—the activity in unstressed cultures.

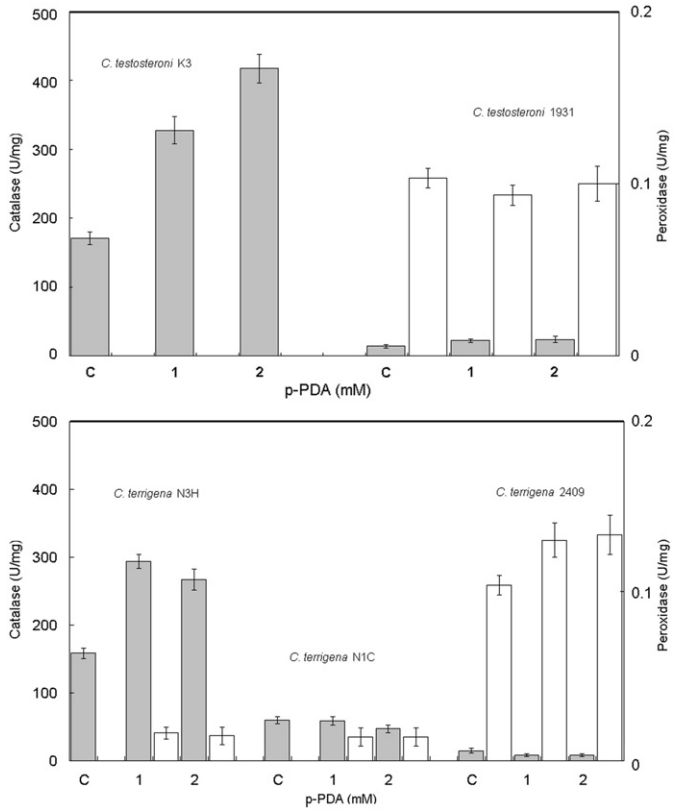

Fig. 8. The effect of *p*-phenylenediamine on the catalase (shaded) and peroxidase (unshaded) activities of *Comamonas* spp. at the middle of exponential phase. Control (C)—activity in unstressed cultures.

all in their absence (Fig. 7). On the other hand, *p*-phenylenediamine (1 and 2 mM) did not stimulate peroxidatic activity in *C. testosteroni* CCM 1931. The catalase activity of the *C. terrigena* N3H isolate was only stimulated by positional isomer *p*-PDA and not by *o*-PDA (Fig. 8).

4. Discussion

A gradual increase in the total catalase activity found in growing cultures of microbial isolates is not surprising. It may arise from an accumulation of endogenous oxidants generated by an increase in respiratory activity. Cells entering stationary phase are subjected to oxidative and starvation stresses and growth may be accompanied by the induction of a new catalase (Lopraset et al., 1996; Jenkins et al., 1988).

The common oxidative stressor hydrogen peroxide is used as a bleaching or microbicidal agent in the paper, food, textile and semiconductor industries. It accumulates in the wastewater and sludges of such industries (Paar et al., 2001). The concentrations of H_2O_2 in these environments are difficult to measure owing to its reactive nature, but Ichise et al. (2008) succeeded in isolating a bacterium *Vibrio rumoiensis* S-1 from these environments, which was able to tolerate 100 mM of H_2O_2. As a result, the concentration of H_2O_2 used here, i.e. 60 mM, is probably not unreasonable. The H_2O_2 tolerance of strain S-1 was attributed to the catalase activity of the cells.

For survival isolates of *C. testosteroni* CCM 1931, *C. testosteroni* K3, *C. terrigena* N3H or N1C and *C. terrigena* CCM 2409, selected largely from polluted environments, the production of catalase and peroxidase activities was important. *C. testosteroni* CCM 1931 and *C. terrigena* CCM 2409 expressed CAT1 band indicating both catalase and peroxidase activities and therefore likely to be catalase–peroxidase (Figs. 2 and 3). Recently, the presence of a gene (*katG*) coding for catalase–peroxidase was confirmed in these cultures (Godočíková et al., 2010). The CAT2 bands of *C. terrigena* CCM 2409 exhibit only peroxidatic activity and probably represent a different type of peroxidase. However, final conclusions on the enzymatic activity and chemical nature of these bacterial hydroperoxidase must await its isolation, purification and molecular characterization (Singh et al., 2008).

A hydroperoxidase variant of the original isolates was generated following their long-term exposure to oxidative stress. These variants gave rise to a considerable diversity of microbial catalase and peroxidase responses to 20 or 40 mM H_2O_2 stress. Boháčová et al. (2006) found that the specific PCR product for catalase-1 in the *C. terrigena* N3H isolate was identical to the corresponding sequence of the *cat-1* gene from *C. terrigena* N1C, which had been isolated from polluted soil, as well. In other strains not exposed to pollution, especially to the reactive oxygen species, this sequence motif could not be found. In spite of this, the different catalases activity in *C. terrigena* N3H and N1C cells can probably be attributed to the regulation of isozyme expression (Fig. 1).

The expression of catalases in response to exogenous stress appears to depend on the concentration of the oxidative stressor as well as the individual organism. Tripathi et al. (2006) showed that catalase and ascorbate peroxidase activities in *Scenedesmus* only increased in 2.5 μM Cu^{+2} and 25 μM Zn^{+2} when oxidative stress was mild and were inhibited in 10 μM Cu^{2+} under intensive oxidative stress. Sabatini et al. (2009) found that *Scenedesmus vacuolatus* microalgae showed a significant increase in both catalase and superoxide dismutase activity in 210 and 414 μM of copper. On the other hand, *Chlorella kessleri* did not show any significant changes in these parameters between 6.2 and 108 μM of copper. Lü et al. (2009) analyzed the superoxide dismutase and catalase activities in the highly quinclora-degrading strain *Stenotrophomonas maltophilia* WZ2 and in *Escherichia coli* K12. Their results indicate that catalase has a much weaker role in defense against quinclorac or bensulfuron-methyl (BSM) induced oxidative stress and that quinclorac is a stronger inducer of superoxide dismutase. Lin et al. (2009) compared the catalase activities, which arise as a response to 0–100 mg L^{-1} BSM exposure in the *B. subtilis* B19, *E. coli* K12 and *B. megaterium* L1 strains. The catalase activity of the *B. megaterium* L1 strain was approximately 10-fold higher than in the other two strains; however, the catalase activity of *B. subtilis* B19 and *E. coli* K12 fell within a similar range.

Our results suggest that catalase is important for the adaptation of cultures to high concentrations (60 mM) of H_2O_2 (Table 2). Electrophoretic analysis also indicates that constitutively expressed catalase (CAT1) represents the major pathway for the resistance of *C. terrigena* N3H cells to toxic peroxides. The isolate can also express a second, larger, and probably more finely regulated type of catalase (CAT2) (Fig. 6).

The appreciable differences in sensitivity to the toxic effect of H_2O_2 treatment between individual isolates and their adapted variants, arising from significantly higher levels of catalases, were observed until the middle of exponential phase, but they were insignificant, at the entry into stationary phase, mainly in the *C. testosteroni* K3 and *C. terrigena* N3H and 2409 strains (Table 1). Resistance at this growth phase can also be influenced by the expression of stress proteins as well. Ferianc et al. (1998) identified 24 proteins induced in the cells of *Vibrio* spp. exposed to cadmium. The majority of them (17) were also expressed during starvation, but only seven proteins were associated exclusively with cadmium stress.

Substituted phenols are ubiquitous contaminants in soil and water. Their presence may result from the degradation of

pesticides and other chemicals that were applied intentionally to soils or from unintentional releases associated with manufacturing processes, production of energy and dyes, and waste disposal (Boyd, 1982). Phenylenediamines (PDA) are listed as one class of these harmful chemical substances. It was expected that to representative PDAs might make good substrates for catalases (Zámocký et al., 2001). The response of microbial catalases to phenylenediamines (PDAs) was diverse. *p*-PDA but not *o*-PDA stimulated catalase in *C. terrigena* N3H (Figs. 7 and 8). *o*-PDA, measurable, significantly stimulated the peroxidatic activity at *C. testosteroni* 1931 (measured using the *o*-dianisidine method), while *p*-PDA stimulated the peroxidatic activity in *C. terrigena* CCM 2409. This specificity can affect microbial growth and the degradability or toxicity of an agent (Barret et al., 1980). We tested the induction of catalases in *Comamonas* cells to environmentally unrealistically high concentrations of PDAs (1–2 mM). The catalase responses observed testify to the likely role of catalases in the high resistance at these isolates to the toxic effect of these pollutants. However, *B. cereus* strain PDa-1, resistant to 5 mM 2-phenylenediamine was isolated from rice-field soil with Takenaka et al. (2006). It seems that microbial isolates from different environments have developed resistance to stresses arising not only from environmental contaminants, but also from extremes of pH as well as other stresses (Bučková et al., 2007).

5. Conclusions

Soil bacteria of the family Comamonadaceae, which are known for their broad catabolic activity in polluted environments, exhibit differences in the expression of catalase and peroxidase activities, most likely due to selective environmental pressure. The diversity of catalases expression in response to exogenous stress appears to depend on the concentration of the oxidative stressor as well as the individual organism. It seems reasonable to suppose that stimulation of peroxidatic activity by 20 and 40 mM H_2O_2 in the *C. testosteroni* CCM 1931 and *C. terrigena* CCM 2409 strains supports metabolic oxidation leading to pollutant decay, whereas the significant increase in catalase activity in the H_2O_2-adapted isolates can be involved in resistance to reactive oxygen species. Diversity of catalases responses to positional *o*- and *p*-isomers of phenylenediamine was also observed. Future investigations will focus on determining the role of catalases in the mechanisms by which specific pollutants can be degraded.

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