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Possible Reasons for Difference in Sensitivity to Oxygen of Two *Escherichia coli* Strains

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Abstract—In preliminary experiments it was found that *Escherichia coli* strains AB1157 and KS400 are different in their abilities to grow under various oxygen levels in cultivation medium: the first strain does not grow under high oxygen conditions, unlike the second one. To investigate whether the damage to cellular components due to production of reactive oxygen species (ROS) was responsible for this difference, the intensity of free radical oxidation of proteins and lipids as well as the activities of selected antioxidant and associated enzymes (superoxide dismutase, catalase, peroxidase, glutathione reductase, glucose-6-phosphate dehydrogenase) were compared in the two strains. The level of thiobarbituric acid-reactive substances was 1.8-2.5-fold higher in AB1157 than in KS400, but the concentration of carbonyl proteins was lower in the AB1157 strain. In both strains growth under higher oxygen levels resulted in higher superoxide dismutase and peroxidase activities in both exponential and stationary phases. Overall, the activities of antioxidant enzymes were always higher in the KS400 strain than in AB1157. The results for both lipid and protein oxidative damage and antioxidant enzyme activities suggest that the differences in oxygen tolerance between these two strains may be due to their different abilities to cope with ROS.

Key words: *Escherichia coli*, oxygen, thiobarbituric acid-reactive substances, carbonyl proteins, antioxidant enzymes

Living organisms when growing aerobically produce reactive oxygen species (ROS) including superoxide anion, hydrogen peroxide, hydroxyl radical, and others as byproducts of dioxygen metabolism [1-4]. When the rate of ROS generation exceeds their decomposition, oxidative stress results. This situation can occur in bacteria when the organisms are exposed to ionizing or near UV-radiation, to some antibiotics and xenobiotics, when they are attacked by phagocytes, etc. [4-7]. Enteric bacteria whose natural niche is anaerobic are subjected to oxidative stress when they leave the intestinal tract and are challenged by sudden exposure to oxygen that results in a burst of superoxide and hydrogen peroxide generation [4, 8, 9].

Most cellular macromolecules are subject to attack by ROS resulting in protein, nucleic acid, carbohydrate, and lipid oxidation. Therefore, all cells possess rather complicated antioxidant defense mechanisms to maintain ROS at appropriate low levels. These include both low molecular weight scavengers such as reduced glutathione (GSH) and specialized enzymes such as superoxide dismutases (SOD), catalases, and peroxidases [7, 9-11]. Most enzymes involved in the adaptive response to oxidative stress in *Escherichia coli* are grouped into the *oxyR* and *soxRS* regulons [9]. Superoxide- and nitric oxide-generating agents activate *soxRS*, and H_2O_2 activates the *oxyR* regulon. The protein products of many genes whose expression is regulated by sensor proteins OxyR and SoxR have clear functions, either direct or indirect, in antioxidant defense. Thus, the *oxyR* regulon switches on catalase–hydroperoxidase I (HPI has both catalase and peroxidase activities), alkyl hydroperoxide reductase, and glutathione reductase (GR), whereas members of the *soxRS* regulon include Mn-SOD, glucose-6-phosphate dehydrogenase (G6PDH), and endonuclease IV [7, 9].

The mentioned regulons can be activated by many agents including paraquat, menadione, ascorbate, and hydrogen peroxide [7-9, 11-13] and provide a good model for understanding defense against ROS excess. In prelim-

Abbreviations: DNPH) 2,4-dinitrophenylhydrazine; G6PDH) glucose-6-phosphate dehydrogenase; GR) glutathione reductase; GSH, GSSG) reduced and oxidized forms of glutathione, respectively; ROS) reactive oxygen species; SOD) superoxide dismutase; TBARS) thiobarbituric acid-reactive substances; TEMED) N,N,N′,N′-tetramethylethylenediamine.

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inary experiments, we found that the widely used *E. coli* strain AB1157, in contrast to strain KS400, was not able to grow under pure oxygen atmospheric conditions. The present study was designed to determine the possible reasons for this difference. Since it could be expected that ROS may be involved, we analyzed various indices of ROS-induced damage to proteins and lipids and the efficiency of antioxidant enzyme systems in both strains under different regimens of oxygen supply.

MATERIALS AND METHODS

Bacteria and chemicals. The strains used in this study were *E. coli* KS400 (wild type K12, *met B*) and AB1157 (wild type K12, F^- *thr-1 leuB6 proA2 his-4 thi-1 argE2 lacY1 galK2 rpsL supE44 ara-14 xyl-15 mtl-1 tsx-33*), kindly provided by Dr. I. Andreeva (Moscow, Russia). All inorganic chemicals were obtained from Reakhim (Russia), and biochemicals were from Reanal (Hungary) and Sigma (USA); chemicals were of the maximum purity available. Medium for bacterial cultivation was from Nutrient Media (Russia).

Growth conditions and cell extracts. Bacteria were grown in a nutrient broth for cultivation of microorganisms containing 10.05 g/liter sprat tryptic hydrolyzate and 4.95 g/liter NaCl. For experimentation, the bacteria were prepared from an overnight (17-18 h) culture grown at 37°C. The resulting bacterial cultures were diluted 1 : 100 (v/v) and grown at the same temperature. Samples of each culture were exposed to each of three different oxygen availability conditions in the cultivation medium: a) limiting oxygen levels (no bubbling); b) bubbling with air (aeration); c) bubbling with pure oxygen (oxygenation). Bacterial growth was monitored by measuring optical density of the culture at 600 nm.

Enzyme activity measurement. Cells were harvested by centrifugation at 3000*g* for 10 min, and washed twice with medium containing 50 mM potassium-phosphate (K-phosphate) buffer (pH 7.0) and 0.5 mM EDTA. After the last centrifugation, the cells were resuspended in the same buffer and disrupted by sonication for 6-8 min using a System UZDN-2T sonicator equipped with a cup horn and operated at 22 kHz. Cell debris was removed by centrifugation at 4000*g* for 10 min at 4°C and cell-free extracts were kept on ice for immediate use.

Suspensions of intact cells were used for catalase activity measurements [14]. Dismutation of hydrogen peroxide by catalase was measured spectrophotometrically at 240 nm with an SF-46 spectrophotometer (LOMO, Russia) using the extinction coefficient for hydrogen peroxide of 39.4 $M^{-1}\cdot cm^{-1}$ [15]. Hydrogen peroxide consumption was assayed in 2 ml of medium containing (as final concentrations) 50 mM K-phosphate buffer (pH 7.0), 0.5 mM EDTA, 10 mM hydrogen peroxide, and 20 µl of cell suspension (about 10^8 cells per ml). Blanks were run in the absence of hydrogen peroxide. One unit of catalase activity was defined as the amount of supernatant protein that utilizes 1 µmol of hydrogen peroxide per minute under the used conditions. Cell-free extracts were used to measure the activities of other enzymes. The peroxidase activity of HPI was evaluated at 436 nm by the modified method of Claiborn and Fridovich [16]. The reaction medium contained 50 mM K-phosphate buffer (pH 7.0), 0.5 mM EDTA, 0.4 mM *o*-dianisidine, 10 mM H_2O_2, 0.3 mM NaN_3, and 20 µl of extract in final volume 2 ml. One unit of peroxidase activity was defined as the extract amount producing a change of 0.01 optical density unit at 436 nm per minute.

The activity of superoxide dismutase was assayed at 406 nm as the inhibition of quercetin oxidation by superoxide anion [17] in reaction medium containing 30 mM Tris-HCl buffer (pH 9.0), 0.3 mM EDTA, 0.8 mM TEMED, 14 µM quercetin, and 3-30 µl of extract in a final volume of 2 ml. One unit of SOD activity was defined as the amount of supernatant protein that inhibited the maximal rate of quercetin oxidation by 50%. The parameter was calculated using the Kinetics computer program [18].

Glutathione reductase (GR) activity was measured by following the consumption of NADPH at 340 nm in reaction medium containing 50 mM K-phosphate buffer (pH 7.5), 0.5 mM EDTA, 1 mM oxidized glutathione (GSSG), 0.25 mM NADPH, and 20 µl of extract in a final volume of 1.5 ml [15]. Two blanks were run—without GSSG and without extract.

The activity of G6PDH was measured by monitoring NADP reduction in reaction medium containing 50 mM K-phosphate buffer (pH 7.5), 0.5 mM EDTA, 0.5 mM $MgSO_4$, 0.2 mM NADP, 1 mM glucose-6-phosphate, and 40 µl of extract in a final volume 2 ml [19]. One unit of GR or G6PDH activity is defined as the amount of supernatant protein that utilizes or produces 1 µmol of NADPH per minute, respectively. All reactions were started by the addition of cell extract and activities were measured at 25°C and expressed per milligram of protein in supernatant.

Determination of carbonyl proteins. The content of carbonyl proteins was evaluated with 2,4-dinitrophenylhydrazine (DNPH). Samples of bacteria after ultrasonication (containing >1.5 mg protein) were treated with 10 mM DNPH in 2 M HCl at a room temperature for 60 min. Blanks contained 2 M HCl without DNPH. Proteins were precipitated by addition of trichloroacetic acid (TCA) to final concentration 10%, centrifuged at 4000*g* for 10 min at 4°C, and washed three times with 1 ml ethanol–ethyl acetate (1 : 1 v/v). The final pellets were dissolved in 6 M guanidine hydrochloride in 5% (v/v) phosphoric acid. Carbonyl content was measured at 370 nm using the extinction coefficient of 22 $mM^{-1}\cdot cm^{-1}$ [20]. The results are expressed in nanomoles per milligram of protein.

Determination of thiobarbituric acid-reactive substances (TBARS). Thiobarbituric acid-reactive substances were measured in cell preparations by a standard procedure [21]. For protein precipitation, 1.0 ml of cell suspension (about 2-3 mg of protein) was supplemented with 1.0 ml 30% TCA in 0.2 M HCl and centrifuged at 4000*g* for 10 min at 4°C; the pellet formed was discarded. The supernatant was mixed with 2.0 ml of a saturated solution of thiobarbituric acid in 0.1 M HCl and 10 mM butylated hydroxytoluene, heated for 60 min at 100°C, cooled, and mixed with 4.0 ml of butanol. The mixture was centrifuged for 10 min at 4000*g*, the organic phase was collected, and the optical density at 535 nm was measured. Results are expressed in nanomoles per milligram of protein using ε_{535} 156 $mM^{-1} \cdot cm^{-1}$ [21].

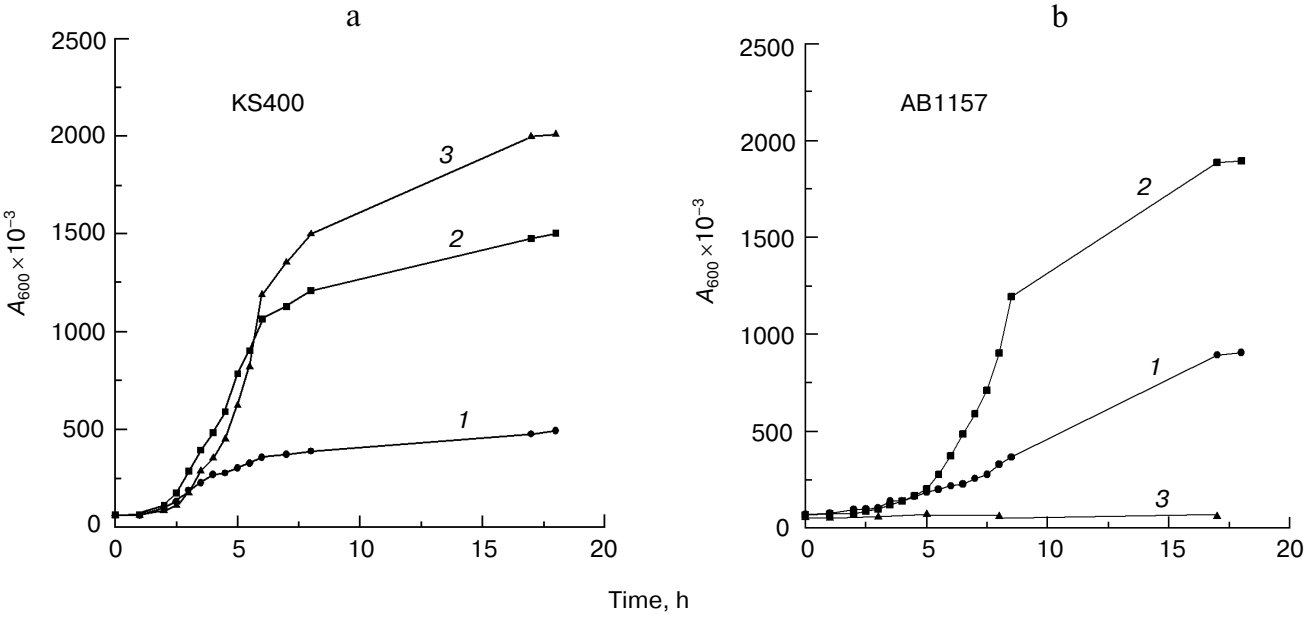
Protein concentration and statistical analysis. Protein concentration was determined by the Coomassie brilliant blue G-250 dye-binding method [22]. Bovine serum albumin was used as the standard. Experimental data are expressed as mean ± SEM, and statistical testing used Student's *t*-test.

RESULTS

The figure (panel (a)) shows the effect of different regimens of cultivation on the growth of the *E. coli* strain KS400. Bacteria cells grown with limited oxygen availability (curve *1*) or with oxygenation (curve *3*) entered the exponential phase of growth after about 3 h, whereas bacteria growing with air bubbling (curve *2*) entered the exponential phase after 2.5 h of growth. The KS400 cultures show different rates of exponential growth with a doubling time of 102 min under limiting oxygen levels, 57 min under aeration, and 44 min under oxygenation, respectively.

The figure (panel (b)) demonstrates growth curves of the *E. coli* strain AB1157 under different oxygen levels in cultivation medium. Under limiting oxygen levels (curve *1*) and aeration (curve *2*) bacteria AB1157 grew more slowly and entered the stationary phase later than the KS400 strain (compare with respective curves in panel (a)). However, AB1157 did not grow under high oxygenation conditions (curve *3*). The length of the lag-phase for this strain was 3 h under aeration and 4 h under limiting oxygen levels. For AB1157 in the exponential phase of growth, the doubling time under limiting oxygen availability was 173 min and for the aerated culture was 79 min.

Table 1 summarizes the numerical characteristics of the growth process for the KS400 and AB1157 strains. The analysis of data presented in the figures and Table 1 indicates that oxygen level dependence of cell growth is not the same for the tested bacterial strains. Both strains demonstrated higher productivity under aeration than limiting oxygen conditions. The KS400 strain productivity under high oxygen conditions occupies position between aeration and limiting oxygen levels. Showing similar growth parameter under limiting oxygen levels and aeration, AB1157 strain demonstrated inability to



Growth curves of *E. coli* KS400 strain (a) and AB1157 strain (b) under different growth conditions: *1*) limiting oxygen levels; *2*) aeration; *3*) oxygenation

Table 1. Effect of oxygen levels on the growth of *E. coli* strains KS400 and AB1157 in exponential and stationary growth periods

Growth phase	Optical density at 600 nm		
	limiting oxygen levels	aeration	oxygenation
KS400			
Exponential (4 h)	0.268 ± 0.008	0.483 ± 0.077[1]	0.353 ± 0.022[1,2]
Stationary (17 h)	0.576 ± 0.055	1.475 ± 0.085[1]	1.995 ± 0.033[1,2]
AB1157			
Exponential (6 h)	0.266 ± 0.061	0.373 ± 0.076	NG
Stationary (17 h)	0.893 ± 0.050	1.887 ± 0.038[1]	NG

Note: Significantly different ($p < 0.05$) from values obtained under limiting oxygen levels conditions ([1]) or aeration ([2]). Data are mean ± SEM (n = 3-4). NG, no growth.

Table 2. Effect of growth conditions on the level of TBARS (nmol/mg protein) and carbonyl proteins (nmol/mg protein) in *E. coli* strains KS400 and AB1157

Parameter	Growth conditions		
	limiting oxygen levels	aeration	oxygenation
KS400			
Exponential			
TBARS	0.100 ± 0.041	0.166 ± 0.030	0.129 ± 0.047
Carbonyl proteins	2.64 ± 0.04	2.11 ± 0.030[1]	2.57 ± 0.03[2]
Stationary			
TBARS	0.097 ± 0.009	0.099 ± 0.024	0.059 ± 0.032
Carbonyl proteins	5.60 ± 0.98*	5.59 ± 1.08*	5.64 ± 1.30
AB1157			
Exponential			
TBARS	0.184 ± 0.061	0.467 ± 0.136[1,a]	NG
Carbonyl proteins	1.94 ± 0.23[a]	3.57 ± 0.53[1,a]	NG
Stationary			
TBARS	0.163 ± 0.025[a]	0.236 ± 0.027[1,a]	NG
Carbonyl proteins	2.27 ± 0.23[a]	1.83 ± 0.09*[,a]	NG

Note: Significantly different ($p < 0.05$) from the corresponding values obtained for the exponential growth phase (*) or from those obtained for the corresponding growth phase under limiting oxygen level ([1]) or aeration ([2]), or from the corresponding parameters obtained for the strain KS400 ([a]). Data are mean ± SEM (n = 3-4). NG, no growth.

grow under oxygenation conditions. This suggested that adaptive mechanisms to high oxygen level in *E. coli* AB1157 are lower than in KS400.

It is possible that the mechanisms responsible for the differences in growth rates under different oxygen conditions are connected to the byproducts of oxygen metabolism, such as ROS. It is well established that ROS oxidize proteins and lipids, producing damage products. Therefore, we further measured two parameters of ROS damage to macromolecules, the amount of protein carbonyls and TBARS in the two bacterial strains that were cultivated under different conditions. Table 2 shows the effect of different growth conditions on the level of carbonyl proteins and TBARS in *E. coli* KS400 and AB1157. The level of TBARS and carbonyl proteins in KS400 depended only slightly upon aeration conditions. The transition from exponential to stationary phase did not significantly change the level of TBARS, but significantly increased 1.5-2.0-fold the amount of carbonyl proteins. For AB1157 in the exponential growth period, the levels

Table 3. Activity of antioxidant and associated enzymes in *E. coli* strain KS400 in exponential and stationary growth period under different cultivation conditions

Enzyme	Growth conditions		
	limiting oxygen levels	aeration	oxygenation
Exponential			
SOD	25.1 ± 4.35	44.9 ± 5.79[1]	97.4 ± 13.4[1,2]
Catalase	18.8 ± 2.88	15.3 ± 1.7	11.6 ± 1.8[1,2]
Peroxidase	0.27 ± 0.02	0.51 ± 0.03[1]	0.62 ± 0.04[1,2]
GR	0.075 ± 0.011	0.079 ± 0.006	0.071 ± 0.012
G6PDH	0.018 ± 0.004	0.024 ± 0.006	0.040 ± 0.008[1]
Stationary			
SOD	44.7 ± 6.5*	85.2 ± 3.6*,[1]	143.9 ± 26.1[1,2]
Catalase	22.2 ± 0.8	41.5 ± 3.6*,[1]	25.8 ± 4.1*,[2]
Peroxidase	0.45 ± 0.02*	0.83 ± 0.14*,[1]	0.87 ± 0.17[1]
GR	0.077 ± 0.012	0.111 ± 0.022	0.115 ± 0.031
G6PDH	0.010 ± 0.002	0.015 ± 0.002	0.028 ± 0.005[1,2]

Note: Significantly different ($p < 0.05$) from the corresponding values obtained for the exponential growth phase (*) or from those obtained for the same growth phase under limiting oxygen conditions ([1]) or aeration ([2]). Data are mean ± SEM (n = 3-4).

of TBARS and carbonyl proteins were higher under aeration conditions in comparison with limiting oxygen availability. Under aeration conditions, AB1157 cells in stationary phase showed 2-fold increased levels of TBARS as compared with exponential culture, but carbonyl proteins did not depend on oxygen concentration. The transition from exponential to stationary phase did not alter the level of TBARS, except for the level of carbonyl proteins under aeration conditions when this parameter decreased almost 2-fold. A comparison of TBARS between the two strains showed that in all cases the absolute levels of TBARS were higher in strain AB1157 than in KS400 cells. Therefore, it may be concluded that intensity of lipid oxidation is much higher under all conditions in the first strain than in the second.

Antioxidant enzymes are one of the most important elements responsible for preventing free-radical modification of cellular components. Activities of antioxidant and associated enzymes (SOD, catalase, peroxidase, GR, and G6PDH) in cells of the two bacterial strains under different cultivation regimens are shown in Tables 3 and 4. In KS400 cells (Table 3) the transition to stationary phase resulted in increase in all enzyme activities except for G6PDH (its activity remained low in all cases) and GR, which also demonstrated very low activity and did not significantly rise in stationary phase on increase in oxygen level. It is well known that SOD forms the first line of antioxidant defense, and it is one of the most important among adaptive proteins. The activity of SOD demonstrated great changeability under the conditions used. Under higher oxygen conditions, the KS400 strain showed higher SOD activities; compared to limiting oxygen concentration, SOD activities under aeration and oxygenation regimens were 178 and 388% higher, respectively. With extended bacterial growth to 17 h (stationary phase) the same pattern of SOD activity increase with increasing oxygen supply was seen. Another enzyme of the *soxRS* regulon, G6PDH, demonstrated a tendency towards increasing activity with the rise in oxygen levels, but this difference was not statistically significant. Aeration and oxygenation also increased the activity of peroxidase (a member of *oxyR* regulon) in both growth periods. On the other hand, catalase activity was unchanged between the limiting oxygen and aeration conditions during exponential growth, but like peroxidase rose with the transition to stationary phase. However, under oxygenation conditions, catalase activity was lower than that in the aeration and/or limiting oxygen conditions.

Table 4 shows the comparable data on antioxidant and associated enzyme activities in strain AB1157. The transition from exponential to stationary growth period

Table 4. Activity of antioxidant and associated enzymes in *E. coli* strain AB1157 in exponential and stationary growth period under different cultivation conditions

Enzyme	Growth conditions	
	limiting oxygen levels	aeration
Exponential		
SOD	16.3 ± 3.8	31.8 ± 3.4[1,a]
Catalase	14.1 ± 0.5	9.0 ± 1.3[1,a]
Peroxidase	0.17 ± 0.01[a]	0.32 ± 0.05[1,a]
GR	0.045 ± 0.003[a]	0.042 ± 0.002[a]
G6PDH	0.024 ± 0.001	0.034 ± 0.006
Stationary		
SOD	38.3 ± 4.7*	67.0 ± 8.9*[,1]
Catalase	13.6 ± 0.5[a]	34.9 ± 6.3*[,1]
Peroxidase	0.95 ± 0.01*[,a]	1.07 ± 0.06*[,1]
GR	0.062 ± 0.011	0.056 ± 0.012[a]
G6PDH	0.020 ± 0.004[a]	0.024 ± 0.002

Note: Significantly different ($p < 0.05$) from the corresponding values obtained for the exponential growth phase (*) or from those obtained for the corresponding growth phase under limiting oxygen level ([1]) or aeration ([2]), or from the corresponding parameters obtained for the strain KS400 ([a]). Data are mean ± SEM ($n = 4$).

under both cultivation regimens caused enhancement of all enzyme activities except for G6PDH and catalase under limiting oxygen availability. Comparison of enzyme activity dependence on cultivation conditions shows that for SOD it is higher under aeration than under limiting oxygen levels. At the same time, the activity of catalase was even a little lower in exponential phase, but it was almost 3-fold higher under aeration conditions in comparison with limiting oxygen concentration in stationary growth period. In exponential phase, peroxidase demonstrated 2-fold higher activity under aeration than under limiting oxygen conditions and in stationary phase the variation was insignificant. Activity of GR and G6PDH did not show any adaptive changes with alteration of oxygen supply.

Comparison of the enzyme activity data in exponential and stationary growth periods under different oxygen availability (Tables 3 and 4) shows that SOD is the most sensitive to environmental modification—its activity rose with culture transition from exponential to stationary phase and with increase in oxygen supply. Under limiting oxygen levels, catalase activity did not vary with transition to stationary growth period and in stationary phase showed enhancement in parallel with increase in oxygen concentration. However, in exponential phase increase in oxygen availability caused some reduction of catalase activity.

Peroxidase demonstrated a great sensitivity to both culture growth period and regimen of aeration. Activity of GR and G6PDH was at low level and showed negligible variation in response to change of growth phase and oxygen availability. It should seem they are not directly involved in adaptation of the strains to modification of cultivation conditions. Therefore, primary antioxidant enzymes (SOD, peroxidase, and partially catalase) play a crucial role in the adaptation process, and associated enzymes GR and G6PDH are less important for adaptation.

DISCUSSION

Escherichia coli is a widely used model system for the investigation of responses to oxidative stress [4, 9, 11, 12]. In the present study we found that two wild-type strains of *E. coli*, AB1157 and KS400, are different in their abilities to grow under conditions of varying oxygen supply (figure, panels (a) and (b)). Previous studies have documented the correlations between an increase in oxygen concentration, an increase in respiration, and an increase of ROS generation in mitochondria via one-electron O_2 reduction [23, 24]. It is possible that a similar mechanism is employed by bacteria because the respiratory chain in *E. coli* accounts for about 87% of the total cellular generation of H_2O_2 [2]. Accordingly, changes in oxygen levels might be related to the modification of activity of some antioxidant enzymes in order to prevent the development of oxidative stress [3].

In the present study, we have demonstrated that the cellular content of oxidized proteins is less sensitive to oxygen concentration than the level of TBARS, a measure of oxidized lipids. At the same time, the levels of TBARS little depended on the bacterial growth phase, unlike carbonyl proteins. It is possible that oxidized lipids are either more quickly metabolized in stationary phase or that the generally higher activities of antioxidant enzymes in stationary phase reduce the rate of TBARS formation and/or accumulation.

We studied here the enzymatic systems that deal with ROS metabolism directly or indirectly. Superoxide dismutase metabolizes superoxide anion and catalase and peroxidase detoxify hydrogen peroxide. GR and G6PDH are auxiliary enzymes that restore the pool of GSH: GR catalyzes the reduction of GSSG using NADPH generated by G6PDH. The activity of primary antioxidant (SOD, peroxidase) and associated (GR, G6PDH) enzymes depended on the oxygen level in the cultivation medium. The enzymes that are members of *soxRS* regulon were more sensitive to environmental oxygen conditions than those of the *oxyR* regulon. SOD activity was higher in both strains under higher oxygen levels in both exponential and stationary growth phases. SOD activity was also higher in both strains when sampled in stationary phase than that in the corresponding exponential phase. Thus, one can conclude that the *soxRS* regulon plays an important role in the defense of *E. coli* against cell damage by elevated environmental oxygen. This is probably connected with superoxide anion production [25].

Catalase activity was reduced at high oxygen levels in KS400 bacteria sampled under both exponential and stationary growth conditions (Table 3). An inactivation of catalase by superoxide resulting from elevated oxygen concentrations may be responsible for this decay [1, 26]. Although the HPII catalase of *E. coli* is not sensitive to O_2^- [27], its low activity in cells from exponential culture would not be sufficient to compensate for inactivation of HPI. The elevation of the peroxidase activity of HPI in aerobic cultures leads us to conclude that OxyR is activated by oxygen. Tables 3 and 4 show that catalase activity is increased by O_2 in bacteria growing in the stationary phase.

Previous studies have linked the elevation of catalase activity caused by oxygen in the stationary phase with HPII synthesis regulated by RpoS (σ^S), whereas expression of OxyR is negligible in this growth period [28-31]. At the same time, the peroxidase activity of HPI is also stimulated by oxygen at the transition to stationary phase. This can be connected with RpoS regulation (HPI is a member of both the *oxyR* and the *rpoS* regulons). It is possible that oxygen activates OxyR in exponential and RpoS in stationary periods, respectively.

One more interesting observation can be made analyzing Tables 3 and 4. Culture transition from exponential to stationary phase does not change the catalase activity in bacteria growing under limiting oxygen levels. These results do not confirm the idea that anaerobiosis causes catalase induction in *E. coli* [32].

The effects of changing oxygen levels on antioxidant enzyme activities were similar in both strains of *E. coli*. However, the absolute values of these activities differed considerably between the two strains. Mean activities of antioxidant and associated enzymes of *E. coli* KS400 were typically 1.5-2.0-fold higher than those of AB1157, except for G6PDH. These results generally correlated inversely with the TBARS contents of the two strains (Table 2). It is supposed that *E. coli* AB1157 is more sensitive to aeration since TBARS quantities in bacteria of this strain were strongly elevated in response to an increase in environmental oxygen level under both growth conditions.

It can be concluded that *E. coli* KS400 demonstrated a more effective antioxidant defense system than that of *E. coli* AB1157. This allows KS400 to retain the necessary cellular oxidant status and makes growth of this strain possible under high oxygenation conditions.

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