TÜBİTAK

Turkish Journal of Biology

http://journals.tubitak.gov.tr/biology/

Research Article

Turk J Biol
(2014) 38: 502-509
© TÜBİTAK
doi:10.3906/biy-1312-93

Study of exogenous oxidative stress response in *Escherichia coli*, *Pseudomonas* spp., *Bacillus* spp., and *Salmonella* spp.

Ifratun NUR, Mohammad Sakil MUNNA, Rashed NOOR*
Department of Microbiology, Stamford University Bangladesh, Dhaka, Bangladesh

Received: 30.12.2013 • **Accepted:** 10.04.2014 • **Published Online:** 11.06.2014 • **Printed:** 10.07.2014

Abstract: With a previous observation of *Escherichia coli* growth cessation with the supplementation of 3 mM hydrogen peroxide (H_2O_2) at the late log phase, the current study further demonstrated the consequences of the addition of an increased concentration (6 mM) of H_2O_2 and further extended the investigation on such an oxidant's impact on the growth of *Salmonella* spp., *Pseudomonas* spp., and *Bacillus* spp. Cell culturability was measured through the enumeration of colony-forming units (CFUs) on agar plates for up to 72 h. Subsequent changes in cell morphology and arrangements were monitored, and the cell viability was simultaneously retraced by spot tests. A sharp decline in the culturable cells of *E. coli* was observed after 48 h with a large mass of cell aggregates upon addition of H_2O_2, while *Pseudomonas* spp. lost viability after 36 h. Impaired morphology of such stressed cells was comparable to those of the untreated cells. Notably, *Pseudomonas* cells were more prone to oxidative damage compared to *E. coli*. In contrast, the impact of H_2O_2 was insignificant in the case of *Salmonella* spp. and *Bacillus* spp., suggestive of a stringent defense mechanism against oxidative stress.

Key words: *Escherichia coli*, *Salmonella* spp., *Pseudomonas* spp., *Bacillus* spp., oxidative stress, hydrogen peroxide, cell viability

1. Introduction

Stress responses among microbial populations against an array of environmental physicochemical stimuli including temperature, irradiation, pH, osmolyte/salt concentration, reactive oxygen species (ROS), redox state, toxic compounds, and nutrient depletion are well known (Türkel, 2000; Den Besten et al., 2009; Ju and Parales, 2010; Fuchs et al., 2011; Kivisaar, 2011; Parry et al., 2011; Deepika et al., 2012; Den Besten et al., 2013; Noor et al., 2013). The ability of bacteria to perceive and respond to these factors is imperative for their survival. Oxidative stress in bacteria commences when the bacteria encounter elevated levels of ROS, such as superoxide anions (O_2^-), which may interact in a number of enzymatic as well as spontaneous chemical reactions to produce more highly reactive oxygen derivatives, such as hydrogen peroxide (H_2O_2) and hydroxyl radicals (•OH). Autoxidation of reduced FAD or reduced flavoprotein gives rise to •OH, and the enzyme NADPH oxidase can also generate O_2^- and H_2O_2 (Cabiscol et al., 2000; Moat et al., 2002). The reaction of O_2^- with nitric oxide forms peroxynitrite anion, which is highly reactive with certain amino acid residues of proteins, which in turn induces the formation of other toxic derivatives within bacterial cells (Squadritoa et al., 1998; Cabiscol et al., 2000; Schutter and Dick, 2000; Moat et al., 2002; Kamat, 2006; Iovine et al., 2008).

To deal with oxidative stress, bacteria are known to evolve protective responses including the peroxidase-, catalase-, and superoxide dismutase (SOD)-mediated detoxification of ROS intermediates and repair of oxidative damage to bacterial DNA (Rosner and Storz, 1997; Desnues et al., 2003; Noor et al., 2009a, 2009b; Murata et al., 2012; Munna et al., 2013; Nagamitsu et al., 2013). In addition, *Escherichia coli* produces cytoplasmic Mn-SOD (*sod*A) and Fe-SOD (*sod*B), which protect DNA and proteins from oxidation, while periplasmic Cu/Zn-SOD (*sod*C) protects the periplasmic and membrane constituents from exogenous O_2^- (Cabiscol et al., 2000; Moat et al., 2002; Rensing and Grass, 2003; Wang et al., 2007). Among other bacteria, *Salmonella* spp. have been reported to induce the soxRS regulon (Straaten et al., 2004; Prieto et al., 2006), while *Pseudomonas* spp. elicit self-generated redox-cycling compounds encoded by *pqrCBAR* genes (Rungrassamee, 2008; Rungrassamee et al., 2009). In *Bacillus* spp., *perR* and *ohr* (Friedberg et al., 1995; Helmann et al., 2003; Zuber, 2009) have been reported to be induced in response to oxidative stress.

The alteration in cellular homeostasis and culturability due to oxidative stress has been widely studied in *E. coli*, where ROS were found to elicit the formation of viable but nonculturable (VBNC) cells in the early stationary

* Correspondence: noor.rashed@yahoo.com

phase, which were further subjected to a σ^E-dependent lysis (Dukan and Nystrom, 1999; Kabir et al., 2004; Nystrom, 2005; Noor et al., 2009b). Earlier observation of the decline of cell viability in the *E. coli* W3110 strain after the addition of 3 mM H_2O_2 led us to examine the similar influence on a different strain of *E. coli* in our laboratory (Kabir et al., 2004; Munna et al., 2013). A simulation of such a stress-responsive event, with different variables like the increased concentration of H_2O_2, the phase of addition of the oxidant, or the response in other strains or species, would largely aid the overall understanding of the cellular responses. Along these lines, the present study was designed to examine the effect of an increased concentration of H_2O_2 (6 mM) in our laboratory stock culture of *E. coli* and subsequently to compare the results with those of other bacteria including *Salmonella* spp., *Pseudomonas* spp., and *Bacillus* spp.

2. Materials and methods

2.1. Bacterial stain, medium, and culture condition

Laboratory stock cultures of *E. coli* SUBE01, *Salmonella* spp. SUBS01, *Pseudomonas* spp. SUBP01, and *Bacillus* spp. SUBB01 were used in this study. Nutrient agar (NA) and nutrient broth (NB) (Hi-Media Laboratories Pvt. Ltd., India) were used for the assay of culturability (Munna et al., 2013). After 24 h of incubation on NA plates at 37 °C, 1 loopful of each of the bacterial cultures was introduced into 5 mL of NB followed by shaking at 100 rpm at 37 °C for 4–6 h (preculture). After adjusting the optical density of the preculture to 0.1 at 600 nm (OD_{600}), 30 µL of each was introduced into 2 different 30-mL sets of NB and incubated at 37 °C with shaking at 100 rpm. After 10 h of growth, 6 mM H_2O_2 was aseptically added into 1 set of nutrient broth and the other set without H_2O_2 was considered as the control (Kabir et al., 2004; Munna et al., 2013). At every 12-h interval, cell growth was monitored by measuring OD_{600}, and the formation of colony-forming units (CFUs) was estimated by counting the colonies up to 72 h at every 24-h interval (Noor et al., 2009b; Munna et al., 2013; Noor et al., 2013). All the experiments were executed 3 times. Statistical analysis regarding bacterial growth was performed by determining the P-value through a t-test. Standard deviations were also measured.

2.2. Microscopy

For the observation of cell morphology and arrangements, simple staining (Crystal Violet, Hucker's Solution) was applied as previously done (Munna et al., 2013). An aliquot of 10 µL of each bacterial suspension was withdrawn at 12-h intervals and the shape and organization of cells were observed under light microscope (Optima Biological Microscope G206, manufactured in Taiwan) at 1000× magnification (Munna et al., 2013).

2.3. Spot test

As described previously, each of the bacterial culture suspensions was serially diluted in 9 mL of NB to obtain up to a 10^{-4} fold dilution (Munna et al., 2013; Noor et al., 2013). From each dilution, an aliquot of 5 µL was dropped onto the NA plates, dried off for 15 min, and incubated at 37 °C for 24 h. Spotting was done after every 12 h of growth (Munna et al., 2013; Noor et al., 2013).

To demonstrate the extent of H_2O_2 resistance in the tested bacteria, the minimal inhibitory concentration (MIC) was evaluated through dilution susceptibility testing in Mueller Hinton broth (Oxoid Ltd., UK) with different concentrations of H_2O_2. The lowest concentration that could inhibit the bacterial growth as indicated by the absence of turbidity was considered as the MIC.

3. Results and discussion

Together with extensive work on temperature stress or nutrient starvation, oxidative stress is of prime interest for molecular biologists. Our earlier work created oxidative stress in bacteria by increasing the amount of ROS, resulting in an elevated level of RNA polymerase σ^E factor and a concomitant effect on cell viability with the accumulation of aggregated proteins (Noor et al., 2009b). Such a finding was consistent with previous suggestive data (Dukan and Nystrom, 1999; Desnues et al., 2003; Cuny et al., 2005; Nystrom, 2005) and we continued research to assess the impact of such stress on other bacterial species (Munna et al., 2013). In our former experiments, apart from the W3110 strain, our laboratory *E. coli* strain also showed the typical stress response towards heat shock (Noor et al., 2013). Furthermore, we subjected a similar strain to external oxidative stress using 3 mM H_2O_2 and observed the stressed cells that lost culturability with morphological defects (Munna et al., 2013). The results of our current study indeed portrayed a phenotypic comparison among an array of bacterial species in response to external oxidative stress imposed by 6 mM H_2O_2.

3.1. Growth retardation of *E. coli* and *Pseudomonas* spp. upon H_2O_2 treatment while *Salmonella* spp. and *Bacillus* spp. remained uninfluenced

Flat colonies typical of stressed ones (Nitta et al., 2000) were observed for *E. coli* cells upon prolonged exposure to 6 mM H_2O_2. After 36 h of incubation, with a concomitant decrease in cell turbidity, most of the colonies of *E. coli* showed flatness followed by the complete elimination of CFUs onward (Figures 1a and 1b). Upon addition of 6 mM H_2O_2, an increased trend of accumulation of cell aggregates in the culture media was noticed, suggestive of lysis of the defective, dead, or VBNC cells formed after 36 h (Figure 1b). While a sharp drop was observed in CFUs, a relatively lower reduction in cell turbidity was noticed, probably due to gradual accretion of the cell aggregates.

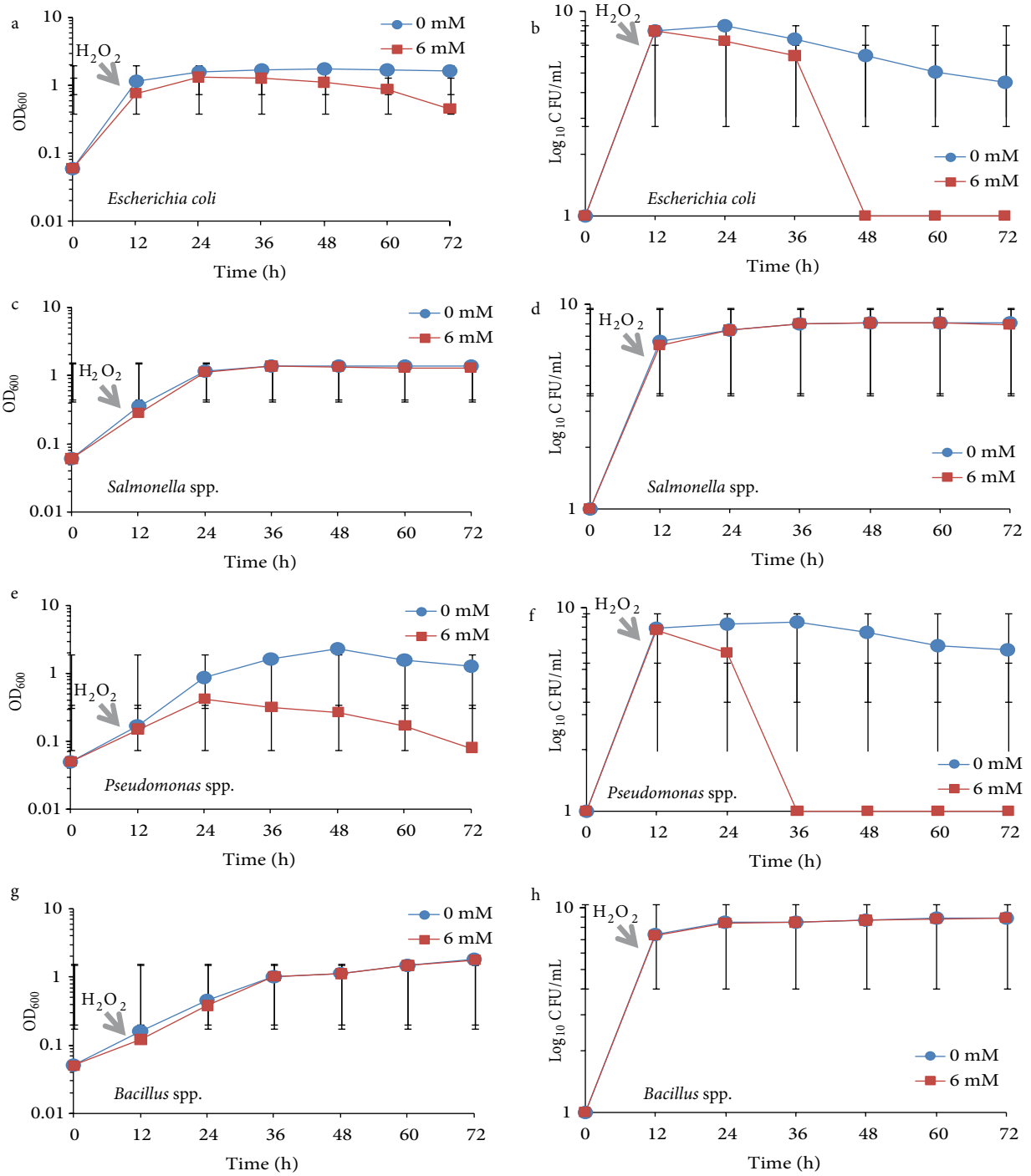

Figure 1. Assessment of cell culturability through the examination of growth of *Escherichia coli* (a, b), *Salmonella* spp. (c, d), *Pseudomonas* spp. (e, f), and *Bacillus* spp. (g, h) upon treatment with 6 mM H_2O_2. Bacterial cells were grown in nutrient broth and 6 mM H_2O_2 was applied after 12 h of bacterial growth. Growth retardation was observed for *E. coli* and *Pseudomonas* spp. after 36 and 24 h, respectively, while the growth of *Salmonella* spp. and *Bacillus* spp. was found to be unaffected by the H_2O_2 treatment. Standard deviations for all data are indicated by error bars.

In the case of *Salmonella* spp., no alteration in the colony phenotypes (i.e. flatness) was noticed, revealing the nonresponsive trait of this bacterium against external oxidative stress. Shiny convex colonies with entire margins were observed at up to 72 h of incubation, which remained uninterrupted. Consistently, unlike *E. coli* cells, abolition

of culturable cells in the case of *Salmonella* spp. was not observed; rather, a steady growth was monitored all along the incubation period with a colony-forming ability of up to 10^8 CFU/ mL after 72 h (Figures 1c and 1d).

Compared to *E. coli*, a relatively quicker drop in culturable cells was observed in *Pseudomonas* spp. with a concomitant reduction in cell turbidity (Figures 1e and 1f). Such a rapid decline may indicate the sensitivity of our laboratory strain of *Pseudomonas* spp. SUBP01 irrespective of its common defense mechanism, as reported earlier (Rungrassamee, 2008; Rungrassamee et al., 2009). On the contrary, like *Salmonella* spp., growth of *Bacillus* spp. was found to be unaffected by the H_2O_2 treatment (Figures 1g and 1h). All the data found in this study were estimated as significant ($P < 0.1$).

3.2. Morphological changes in *E. coli* and *Pseudomonas* cells

In contrast to *E. coli* and *Pseudomonas* cells, the relatively inert response of *Salmonella* spp. and *Bacillus* spp. against H_2O_2 led to further examination of cell morphology and arrangements, with the objective of achieving a detailed view of the oxidative stress response in the former cells. For this purpose, cell morphology of all 4 bacterial species was observed under light microscopy after the addition of 6 mM H_2O_2 (Figure 2).

As in the case of *E. coli* and *Pseudomonas* cells, longer cells were observed after 36 h of incubation, which were significantly comparable not only with the control strains (i.e. untreated with H_2O_2) but also with the H_2O_2-charged *Salmonella* and *Bacillus* cells. Interestingly, in comparison to *E. coli*, *Salmonella*, and *Bacillus* cells, *Pseudomonas* cells quickly elongated just after the addition of H_2O_2 at 12 h (Figures 2a–2l′).

Another observation of *E. coli* cells was the transformation of the longer cells to comparatively shorter and nearly spherical shapes, as well as the formation of the cell aggregates after 72 h with a higher frequency of formation of aggregated cells in the 6 mM H_2O_2-treated

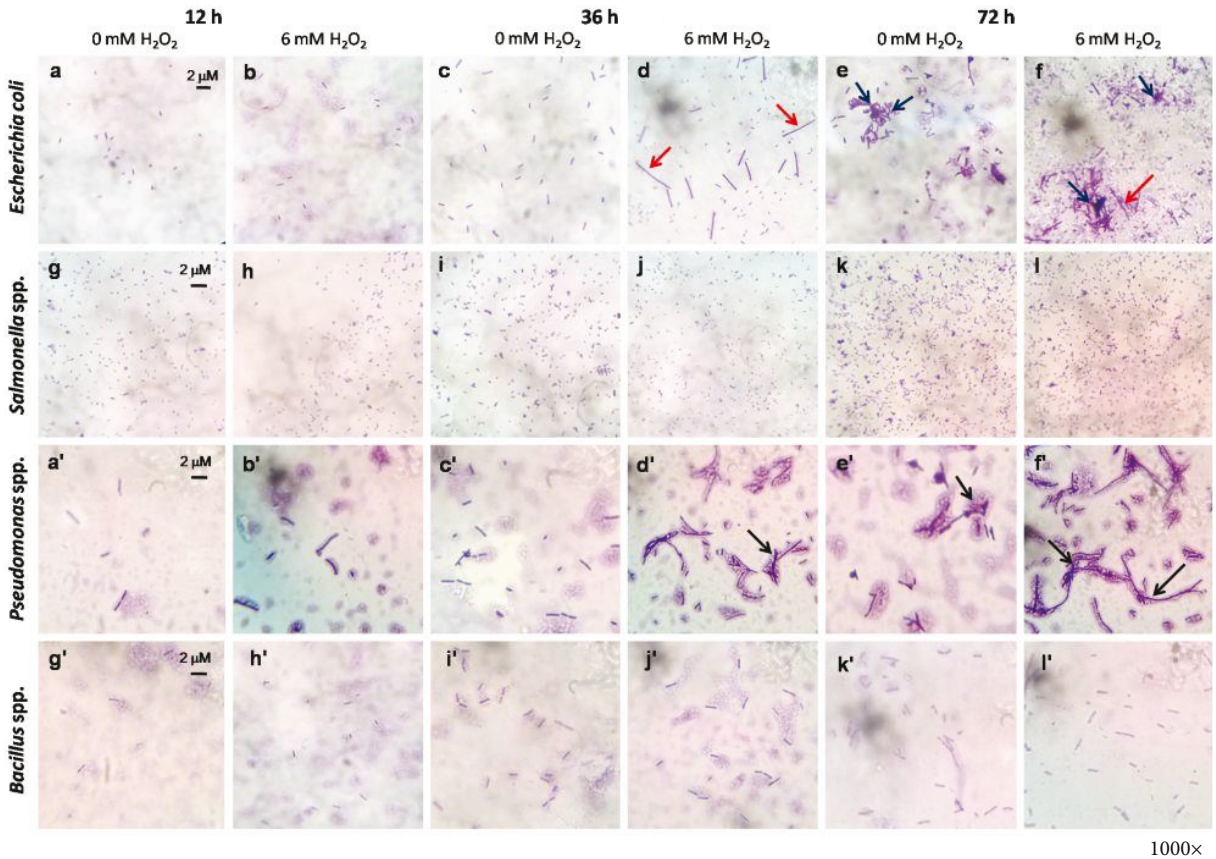

Figure 2. Morphological examination of *Escherichia coli* (a–f), *Salmonella* spp. (g–l), *Pseudomonas* spp. (□′–f′), and *Bacillus* spp. (g′–h′) upon 6 mM H_2O_2 treatment to the bacterial culture media. Bacterial cells were grown in nutrient broth and 6 mM H_2O_2 was applied after 12 h of bacterial growth. Interestingly, cells were found to be sharply elongated just after the addition of H_2O_2 for *Pseudomonas* spp. Such morphological change was also observed in *E. coli* after 36 h. *Salmonella* spp. and *Bacillus* spp. were found to be unaffected regardless of the H_2O_2 treatment.

culture media. However, compared to the stressed cells of *E. coli*, *Pseudomonas* cells were found to be stretched to a greater extent on prolonged incubation under the oxidative stress. In contrast, no such morphological or arrangement changes were observed for *Salmonella* or *Bacillus* cells regardless of H_2O_2 treatment.

3.3. Confirmative demonstration of *E. coli* and *Pseudomonas* viability loss

The sharp decline in culturable cell population fraction, as well as the morphological changes due to the exogenous oxidative stress (imposed by 6 mM H_2O_2 treatment) in *E. coli* and *Pseudomonas* spp. (Figures 2a–2l′), in contrast to *Salmonella* and *Bacillus* cells, led to further cross-checks of the stressed physiology of the cells through spot tests. As shown in Figure 3, after 36 h of growth of *E. coli* was found to be significantly inhibited compared to the control strain (untreated with 6 mM H_2O_2). After 72 h, the bacterium treated with 6 mM H_2O_2 was completely unable to form colonies, consistent with the earlier growth studies. Similar to the morphological observations, growth of *Pseudomonas* cells was also hindered even after 12 h upon the addition of 6 mM H_2O_2. Conversely, irrespective of the H_2O_2 treatment, *Salmonella* spp. and *Bacillus* spp. continued to form colonies; the nonreactivity of these cells against H_2O_2 was confirmed by their growth sustainability (Figure 3).

While oxidative stress responses have been well investigated in *E. coli* and *Pseudomonas* spp., a thorough query on such signal transduction pathways and the stress response in other bacteria (such as *Salmonella* spp., *Bacillus* spp., etc.) based on the available information about their survival mechanisms has yet to be undertaken (Christman et al., 1985; Inaoka et al., 1990; Farr and Kogoma, 1991; Givskov et al., 1994; Nitta et al., 2000; Sabra et al., 2002; Kabir et al., 2004; Passalacqua et al., 2006, 2007; Den Besten et al., 2009; Noor et al., 2009a, 2009b; Zuber, 2009; Pohl et al., 2011; Huillet et al., 2012; Murata et al., 2012; Den Besten et al., 2013; Nagamitsu et al., 2013). In relation to current studies on bacterial oxidative stress responses, the present study employed preliminary experiments to resolve the impact of increased concentration of the oxidant. However, in comparison to our other recent work (Munna et al., 2013), the present study generated more information on the toxicity of H_2O_2 in *E. coli* cells. They were clearly distinguishable from the cells of *Salmonella* spp., which showed nonresponsive traits, evidence of a distinct defense mechanism possibly involving proteins encoded by the soxRS regulon (Straaten et al., 2004; Prieto et al., 2006). Moreover, study of MICs revealed that *E. coli* and *Pseudomonas* spp. were inhibited by 2 mM and 3 mM concentration of H_2O_2, respectively, while the minimal concentration of H_2O_2 required to inhibit the growth of

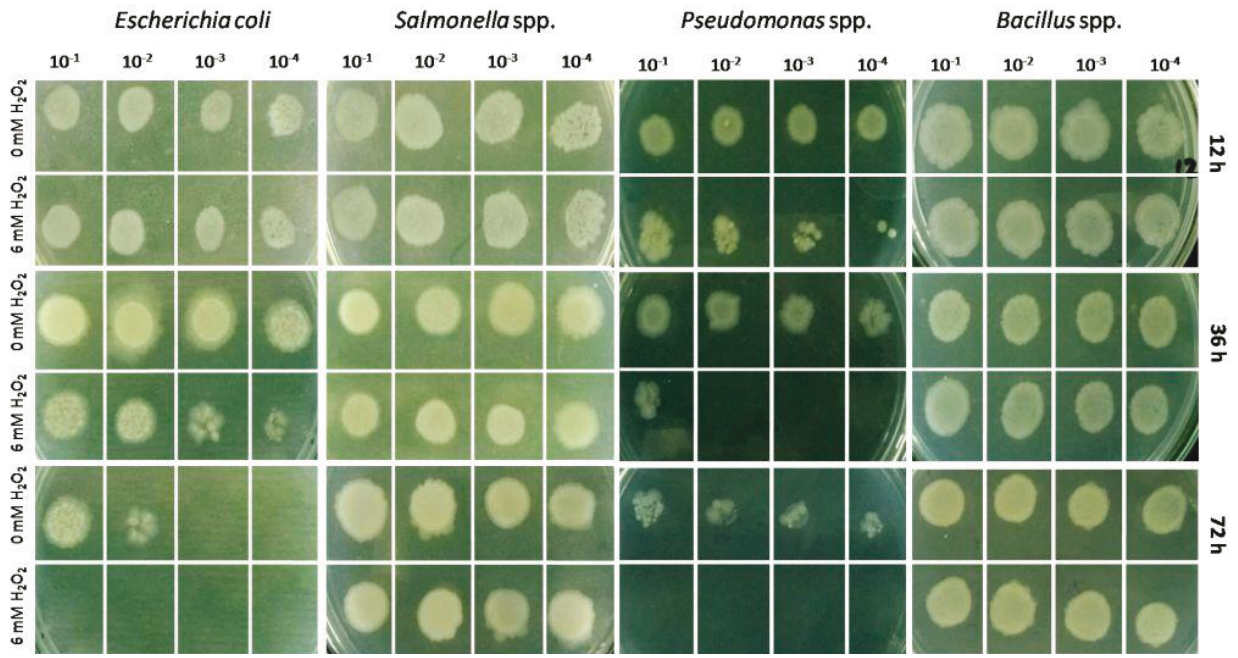

Figure 3. Confirmative demonstration of culturability and survival potential of *Escherichia coli*, *Salmonella* spp., *Pseudomonas* spp., and *Bacillus* spp. cells by spot test after 6 mM H_2O_2 treatments. Bacterial cells were grown in nutrient broth and 6 mM H_2O_2 was applied after 12 h of bacterial growth. In agreement with previous observations, growth retardation was observed for *Pseudomonas* spp. just after the addition of H_2O_2 after 12 h of growth. The growth of *E. coli* was found to be affected after 36 h upon the addition of H_2O_2. As previously observed, *Salmonella* spp. and *Bacillus* spp. remained unaffected.

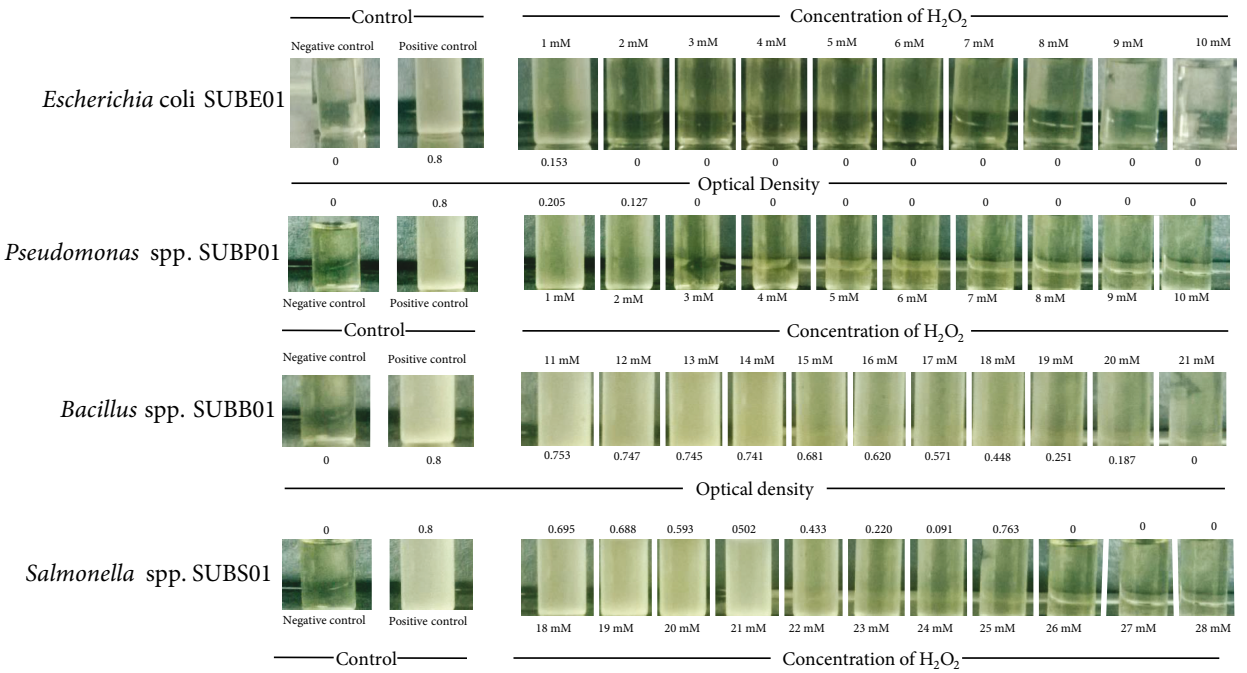

Figure 4. Assessment of MICs of H_2O_2 for *E. coli* (SUBE01), *Pseudomonas* spp. (SUBP01), *Bacillus* spp. (SUBB01), and *Salmonella* spp. (SUBS01). Dilution susceptibility test in Mueller Hinton broth was employed. The MIC of H_2O_2 was 2 mM for *E. coli*; for *Pseudomonas* spp., it was 3 mM; for *Bacillus* spp. it was estimated as 21 mM; and for *Salmonella* spp. SUBS01, it was 26 mM.

Bacillus spp. and *Salmonella* spp. was found to be 21 mM and 26 mM, respectively (Figure 4). Such a comparative approach is indeed new to this field. However, the current investigation focused only on the phenotypic changes upon oxidant stimulation; an extension of the study investigating genetic regulation along with assaying glutathione, protein carbonylation, intracellular oxidation levels, activity of the antioxidant enzymes such as superoxide dismutase, catalase, etc. and comparative analysis of H_2O_2's effects with those of other oxidative stress agents such as menadione would further support the data obtained here.

Nevertheless, our data clearly showed the physiological influence of external oxidative stress in *E. coli* and *Pseudomonas* spp., which is in agreement with our previous studies (Kabir et al., 2004; Munna et al., 2013), and provided new information on the defense strategies of *Salmonella* spp. and *Bacillus* spp. against oxidative stress. *Salmonella* spp. and *Bacillus* spp. formed CFUs with the absence of aggregates in culture media, unlike the *E. coli* cells. The cell morphology of the former strain was also found to be completely unaffected by the oxidant, which supported the maintenance of cell culturability of *Salmonella* spp.

In conclusion, the current study detailed the phenotypic changes in a range of bacteria as a result of oxidative stress, which is in agreement with the existing knowledge on the genetic level of oxidative stress regulation. The comparative oxidative stress responses among the 4 bacterial species studied in the current investigation would be of significance in terms of cellular survival strategy. Despite the lack of molecular study of stress-responsive elements, our study revealed the phenotypic changes in a heterogeneous population of bacteria caused by external oxidative stimulation. The presented results may provide further information on the triggering phase of oxidative stress commencement, rate of damage at cellular level, and, finally, the consequences of VBNC formation in a large array of other bacterial populations.

Acknowledgment

We thank Stamford University Bangladesh for proving laboratory facilities and technical and financial support.

References

Cabiscol E, Tamarit J, Ros J (2000). Oxidative stress in bacteria and protein damage by reactive oxygen species. Int Microbiol 3: 3–8.

Christman MF, Morgan RW, Jacobson FS, Ames BN (1985). Positive control of a regulon for defenses against oxidative stress and some heat-shock proteins in *Salmonella typhimurium*. Cell 41: 753–762.

Cuny C, Dukan L, Fraysse L, Ballesteros M, Dukan S (2005). Investigations of the first events leading to the loss of culturability during *Escherichia coli* starvation: future nonculturable bacteria form a subpopulation. J Bacteriol 187: 2244–2248.

Deepika G, Karunakaran E, Hurley CR, Biggs CA, Charalampopoulos D (2012). Influence of fermentation conditions on the surface properties and adhesion of *Lactobacillus rhamnosus* GG. Microb Cell Fact 11: 116.

Den Besten HMW, Effraimidou S, Abee T (2013). Catalase activity as a biomarker for mild stress-induced robustness in *Bacillus weihenstephanensis*. Appl Environ Microb 79: 57–62.

Den Besten HMW, Mols M, Moezelaar R, Zwietering MH, Abee T (2009). Phenotypic and transcriptomic analyses of mildly and severely salt-stressed *Bacillus cereus* ATCC 14579 cells. Appl Environ Microb 75: 4111–4119.

Desnues B, Cuny C, Gregori G, Dukan S, Aguilaniu H, Nystrom T (2003). Differential oxidative damage and expression of stress defense regulons in culturable and nonculturable *Escherichia coli* cells. EMBO Rep 4: 400–404.

Dukan S, Nystrom T (1999). Oxidative stress defense and deterioration of growth arrested *Escherichia coli* cells. J Biol Chem 274: 26027–26032.

Farr SB, Kogoma T (1991). Oxidative stress responses in *E. coli* and *Salmonella typhimurium*. Microbiol Rev 55: 561–585.

Friedberg EC, Walker GC, Siede W (1995). DNA Repair and Mutagenesis. 2nd ed. Washington DC, USA: ASM Press.

Fuchs G, Boll M, Heider J (2011). Microbial degradation of aromatic compounds - from one strategy to four. Nat Rev Microbiol 9: 803–816.

Givskov M, Eberl L, Moller S, Poulsen LK, Molin S (1994). Responses to nutrient starvation in *Pseudomonas Putida* KT2442: analysis of general cross-protection, cell shape, and macromolecular content. J Bacteriol 176: 7–14.

Helmann JD, Wu MFW, Gaballa A, Kobel PA, Morshedi MM, Fawcett P, Paddon C (2003). The global transcriptional response of *Bacillus subtilis* to peroxide stress is coordinated by three transcription factors. J Bacteriol 185: 243–253.

Huillet E, Tempelars MH, Andre-Leroux G, Wanapaisan P, Bridoux L, Makhzamis S, Panbangred W, Martin-Verstraete I, Abee T, Lereclus D (2012). PIcRa, a new quorum-sensing regulator from *Bacillus cereus*, plays a role in oxidative stress response and cysteine metabolism in stationary phase. PLoS ONE 7: e51047.

Inaoka T, Matsumura Y, Tsuchido T (1990). SodA and manganese are essential for resistance to oxidative stress in growing and sporulating cells of *Bacillus subtilis*. J Bacteriol 181: 1939–1943.

Iovine M, Nicole P, Seema A, Voldman G, Wasserman MJ, Blaser, Weinrauch Y (2008). Reactive nitrogen species contribute to innate host defense against *Campylobacter jejuni*. Infect Immun 76: 986–993.

Ju KS, Parales RE (2010). Nitroaromatic compounds, from synthesis to biodegradation. Microbiol Mol Biol R 74: 250–272.

Kabir MS, Yamashita D, Noor R, Yamada M (2004). Effect of σ^S on σ^E-directed cell lysis in *Escherichia coli* early stationary phase. J Mol Microb Biotechnol 8: 189–194.

Kamat JP (2006). Peroxynitrite: a potent oxidizing and nitrating agent. Indian J Exp Biol 44: 436–447.

Kivisaar M (2011). Evolution of catabolic pathways and their regulatory systems in synthetic nitroaromatic compounds degrading bacteria. Mol Microbiol 82: 265–268.

Moat AG, Foster JW, Spector MP (2002). Lipids and sterols. In: Microbial Physiology. 4th ed. New York, NY, USA: John Wiley and Sons Inc., pp. 450–464.

Munna MS, Nur I, Rahman T, Noor R (2013). Influence of exogenous oxidative stress on *Escherichia coli* cell growth, viability and morphology. Am J Bioscience 1: 59–62.

Murata M, Noor R, Nagamitsu H, Tanaka S, Yamada M (2012). Novel pathway directed by σ^E to cause cell lysis in *Escherichia coli*. Genes Cells 17: 234–247.

Nagamitsu H, Murata M, Kosaka T, Kawaguchi J, Mori H, Yamada M (2013). Crucial roles of MicA and RybB as vital factors for σ^E dependent cell lysis in *Escherichia coli* long-term stationary phase. J Mol Microb Biotechnol 23: 227–232.

Nitta T, Nagamitsu H, Murata M, Izu H, Yamada M (2000). Function of the σ^E regulon in dead-cell lysis in stationary phase *Escherichia coli*. J Bacteriol 182: 5231–5237.

Noor R, Islam Z, Munshi SH, Rahman F (2013). Influence of temperature on *Escherichia coli* growth in different culture media. J Pure Appl Microb 7: 899–904.

Noor R, Murata M, Nagamitsu H, Klein G, Rain S, Yamada M (2009a). Dissection of σ^E dependent cell lysis in *Escherichia coli*: roles of RpoE regulators RseA, RseB and periplasmic folding catalyst Ppid. Genes Cells 14: 885–899.

Noor R, Murata M, Yamada M (2009b). Oxidative stress as a trigger for growth phase-specific σ^E dependent cell lysis in *Escherichia coli*. J Mol Microb Biotechnol 17: 177–187.

Nystrom T (2005). Role of oxidative carbonylation in protein quality control and senescence. EMBO J 24: 1311–1317.

Passalacqua, K, Bergman N, Lee JY, Sherman D, Hanna P (2006). The superoxide dismutases of *Bacillus anthracis* do not cooperatively protect against endogenous superoxide stress. J Bacteriol 188: 3837–48.

Passalacqua K, Bergman N, Lee JY, Sherman D, Hanna P (2007). The global transcriptional responses of *Bacillus anthracis* Sterne (34F2) and a delta *sod*A1 mutant to paraquat reveal metal ion homeostasis imbalances during endogenous superoxide stress. J Bacteriol 189: 3996–4013.

Pohl S, Tu W, Aldridge P, Gillespie C, Hahne H, Mader U (2011). Combined proteomic and transcriptomic analysis of the response of *Bacillus anthracis* to oxidative stress. Proteomics 11: 3036–55.

Prieto AI, Morales FR, Casadesús J (2006). Repair of DNA damage induced by bile salts in *Salmonella enterica*. Genetics 174: 575–584.

Rensing C, Grass G (2003). *Escherichia coli* mechanisms of copper homeostasis in a changing environment. FEMS Microbiol Rev 27: 197–213.

Rosner JL, Storz G (1997). Regulation of bacterial responses to oxidative stress. Curr Top Cell Regul 35: 163–177.

Rungrassamee W (2008). Oxidative stress responses in *Escherichia coli* and *Pseudomonas aeruginosa*. PhD, University of Massachusetts Amherst, Amherst, MA, USA.

Rungrassamee W, Ryan KC, Maroney MJ, Pomposiello PJ (2009). The PqrR transcriptional repressor of *Pseudomonas aeruginosa* transduces redox signals via an iron-containing prosthetic group. J Bacteriol 191: 6709–6721.

Sabra K, Kim EJ, Zeng AP (2002). Physiological response of *Pseudomonas aeruginosa* PAO1 to oxidative stress in controlled microaerobic and aerobic cultures. Microbiology 148: 3195–3202.

Schutter ME, Dick RP (2000). Comparison of fatty acid methyl ester (FAME) methods for characterizing microbial communities. Soil Sci Soc Am J 64: 1659–1668.

Squadritoa GL, Pryor WA (1998). Oxidative chemistry of nitric oxide: the roles of superoxide, peroxynitrite, and carbon dioxide. Free Radical Bio Med 25: 392–403.

Straaten TVD, Zulianello L, Diepen AV, Granger DL, Janssen R, Dissel JTV (2004). *Salmonella enterica* serovar typhimurium RamA, intracellular oxidative stress response, and bacterial virulence. Infect Immun 72: 996–1003.

Türkel S (2000). Effects of various physiological stresses on transcription of the SUC2 gene in the yeast *Saccharomyces cerevisiae*. Turk J Biol 24: 233–240.

Wang Y, Wang H, Yang CH, Wang Q, Mei R (2007). Two distinct manganese-containing superoxide dismutase genes in *Bacillus cereus*: their physiological characterizations and roles in surviving in wheat rhizosphere. FEMS Microbiol Lett 272: 206–213.

Zuber P (2009). Management of oxidative stress in *Bacillus*. Annu Rev Microbiol 63: 575–597.