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# Influence of exogenous oxidative stress on *Escherichia coli* cell growth, viability and morphology

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## To cite this article:

Md. Sakil Munna, Ifra Tun NUR, Tasmina Rahman, Rashed Noor. Influence of Exogenous Oxidative Stress on *Escherichia Coli* Cell Growth, Viability and Morphology. *American Journal of BioScience*. Vol. 1, No. 4, 2013, pp. 59-62. doi: 10.11648/j.ajbio.20130104.12

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**Abstract:** The present study attempted to determine the influence of exogenous oxidative stress on the cell viability of *Escherichia coli*. In this regard, 3mM hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) was added to the late log phase of *E. coli* culture, and afterwards the phenotype, cell morphology and the ability to form colony forming units (CFU) on agar plates were examined. As expected, a quick phenotypic suppression as well as a rapid decline in viable and culturable cell numbers was observed at the mid-stationary phase as compared to control. Interestingly, a large mass of cell aggregates was noticed upon addition of H<sub>2</sub>O<sub>2</sub>. Thus the current investigation corroborated the previous findings and further added to the existing knowledge on oxidative stress events in *E. coli*.

**Keywords:** *Escherichia Coli*, Oxidative Stress, Hydrogen Peroxide (H<sub>2</sub>O<sub>2</sub>), Cell Culturability, Cell Death

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## 1. Introduction

Bacterial cellular responses to different stress evoking physical and chemical stimuli have attracted current interest in molecular biology. A range of bacteria, including *E. coli*, *Bacillus sp.* and *Pseudomonas sp.* [1-17] have been reported to elicit an array of survival strategies in response to such changes. Oxidative stress can be efficiently defined as an accumulation of excess pro-oxidants in bacterial cells causing damage to cellular proteins and nucleic acids and hence imparting cell non-culturability or cell death and lysis [8,12,18-22].

Almost all aerobic organisms have evolved complex defense and repair mechanisms against oxidative stress, resulting in the reduction of the reactive oxygen species (ROS), and hence increasing the viable cells [8,9]. It has been demonstrated previously that oxidative stress that accumulated in the transition period gives rise to viable but non-culturable (VBNC) cells. These VBNC cells are in turn lysed by a  $\sigma^E$ -dependent process [6,8,9,13] and  $\sigma^E$ -regulon also causes dead-cell lysis specific to the early stationary phase in addition to its well known role in the extracytoplasmic stress response in *E. coli* [23,24].

Additionally, our recent study on temperature stress on *E. coli* demonstrated a decline in cell viability upon increase in temperature [24]. In a previous study, upon increase in the growth temperature, the level of ROS and the bacterial cell

lysis were found to increase; this may lead to the inference that oxidative stress is a major factor responsible for the VBNC cell formation and cell lysis at the early stationary phase or at elevated temperature [25]. Therefore, investigation of the impact of oxidative stress on *E. coli* cells could be of an interest for a better understanding of the cellular stress responsive strategies.

Along these lines, the present study was designed to investigate the effect of exogenous oxidative stress by employing hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) on our laboratory strain of *E. coli* though observation of the changes in different parameters such as colony morphology, bacterial growth and survival capacity, cell morphology, optical density and accumulation of cellular aggregates in culture media during the incubation period.

## 2. Materials and Methods

### 2.1. Bacterial Strain, Medium and Culture Condition

Bacterial strains used in this study were previously preserved on Nutrient Agar (NA) at 4 °C temperature. The medium used for the growth and subsequent subcultures of the bacteria were Nutrient Agar (NA) and Nutrient Broth (NB). After 24 hour incubation on NA plates at 37°C, one

loopful of colonies was introduced into 5ml NB followed by shaking at 100 rpm at 37 °C for 4 hours (pre-culture). After adjusting optical density of the pre-culture at 600 nm ( $OD_{600}$ ) to 0.1, 30  $\mu$ L was each transferred into 2 different sets of 30 ml of nutrient broth and incubated at 37 °C temperature with shaking at 100 rpm. After 10 hours 3mM  $H_2O_2$  was added into one set of 30 ml of nutrient broth and the other set without  $H_2O_2$  was considered as the control. At every 12 hours intervals cell growth was monitored by measuring OD at 600 nm and the CFUs were estimated by counting the colonies at every 24 hours [8,24].

## 2.2. Spot test

The bacterial suspension was serially diluted in 9 ml nutrient broth to obtain  $10^{-4}$ . From each of the dilutions, 5  $\mu$ L of the bacterial suspension was spotted on the nutrient agar plates and incubated at 37 °C for 24 hours after the plates had dried off. Spotting was done at every 12 hours interval.

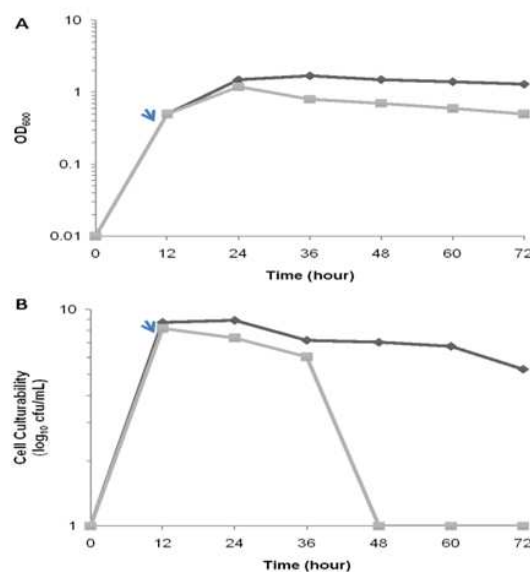
## 2.3. Microscopy

By using simple staining technique, bacterial suspension were drawn at 12 hours intervals and the shape and arrangement of colonies were observed under light microscope (Biological Microscope G206) at 100 $\times$  magnification.

## 3. Results & Discussion

Previous studies have established that a substantial number of *E. coli* W3110 cells become viable but non-culturable at the early stationary phase triggered by the accumulation of reactive oxygen species (ROS) at this phase, most of which are further aimed to cellular lysis [8,23]. Whether such an event is of global consequence for all of the *E. coli* strains is of great interest to understand the global bacterial cellular response cascade. The present study thus fortified these earlier observations employing the laboratory strain of *E. coli* to the simulation in cell growth when exposed to the external oxidative stress.

In the current investigation, we looked for such a stress response event as a cell survival strategy, and our data clearly revealed the lysis of the defective, dead or the VBNC cells (unable to form colonies on plates) observed through the decrease in turbidity of cell culture with a subsequent increase in cell aggregates in the culture medium. Thus the exogenous oxidative stress generated by the addition of 3mM  $H_2O_2$  resulted in substantial impact on *E. coli* cells viability and culturability.



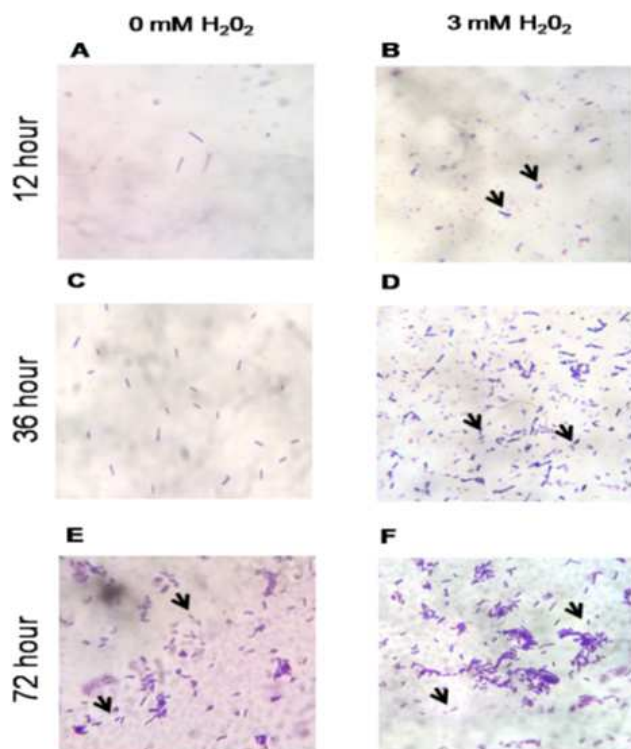
**Figure 1.** Effect of  $H_2O_2$  on growth of *E. coli* in terms of OD (A) and CFU (B) at 12 hours intervals. 3mM  $H_2O_2$  was added at 10 hours of growth as shown by arrowheads

### 3.1. Inhibition of Growth and Viability upon Exogenous Oxidative Stress

An interesting observation of cellular stress was first reported by Nitta *et al.*, [23], emphasizing on the flatness of *E. coli* colonies, which was further corroborated later [8,9]. In agreement with those observations, the current study also showed at the onset of growth of flat colonies of *E. coli* laboratory strains at the stationary phase upon exposure to 3mM  $H_2O_2$  (data not shown). However, no significant changes were observed on colony phenotype in the absence of  $H_2O_2$ . It is interesting to note that while at 36 hour, most of the colonies were flat as stated earlier, further incubation resulted in inhibition of the formation of the CFU, thus revealing the complete elimination of culturable cells by the treatment with  $H_2O_2$  (Fig. 1). This is presumably due to the excess accumulation of ROS caused by the addition of  $H_2O_2$ , which in turn led to the transformation of viable and culturable cells to become VBNC and hence to undergo lysis during the late stationary phase [8].

### 3.2. Impairment of Cell Morphology and Arrangement Due to Exogenous Oxidative Stress

The observation of the flattened colony phenotype further led us to examine the probable morphological changes in the stressed cells as shown in Fig. 1. For this purpose, after addition of 3mM  $H_2O_2$ , the cell morphology was examined under light microscope (Fig. 2).



**Figure 2.** Morphological changes of *E. coli* cells upon  $H_2O_2$  treatment

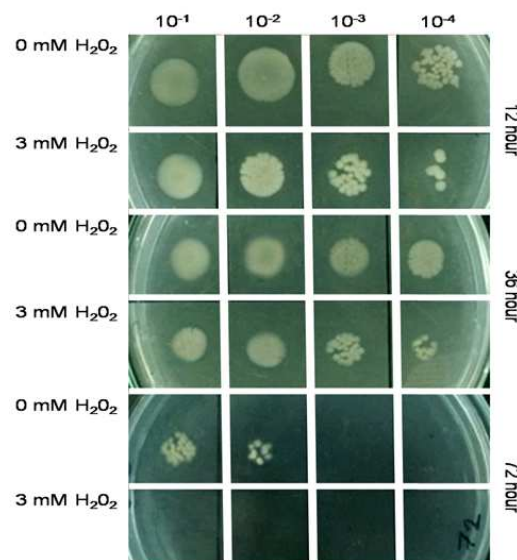
After 36 hour of incubation, most of the cells were found to be disrupted (Fig. 2D), and during 60-72 hours of incubation, these defective cells were observed to be shortened as well as in aggregated arrangement (Fig. 2E) compared to those not exposed to the external stress, i. e., control (Fig. 2A, C & E). Interestingly, the length of cells increased after 36 hours (Fig. 2C) whilst the cells were observed to aggregate at around 60-72 hours (Fig. 2E). This phenomenon is assumed to be the onset of chromosomal DNA segregation of the defective cells prior to loss of their culturability. However, such an observation of impaired morphology and arrangement further led us to confirm the inability of CFU forming capacity of these defective cells.

### 3.3. Confirmation of Loss of Viability and Culturability Due to Exogenous Oxidative Stress

After 36 hours of incubation, stressed cells (as caused by the addition of 3mM  $H_2O_2$ ) were observed to lose the cell viability and culturability as indicated by a relatively lower OD at 600nm and the significant reduction in CFU (Fig. 1). At the same time, the gradual accumulation of cell aggregates in the liquid culture media of the stressed cells was also indicative of cell lysis caused by the external oxidative stress.

As depicted in Fig. 3, the slower growth rate of the stressed cells is supportive to our previous findings of *E. coli* VBNC cells. Considerable growth was observed in control strains (untreated) up to 36 hours of incubation, followed by a relatively slow growth afterwards. In the presence of 3mM  $H_2O_2$ , considerable growth inhibition was observed at 12

hour, and after 36 hour, growth was completely inhibited, in consistent to the data shown in Fig. 1. Such a confirmative experimental approach on growth inhibition by oxidative stress is for the first time reported in context to our previous studies [8,9,24,25].



**Figure 3.** Growth and survival ability of *E. coli* cells through spot test upon  $H_2O_2$  treatment

Despite the lack of molecular investigation in the current investigation, the results presented in our study corroborate our previous reports on cellular homeostasis as well the stress responsive phenotypes [8,9,24-28]. Nevertheless, the finding of this study is not only a confirmation of the previous findings but also is of global importance with regard to the *E. coli* strain used in the laboratory.

## 4. Conclusion

Our study demonstrated the impact of 3mM  $H_2O_2$  on colony phenotype, cell morphology, cell viability and culturability of *E. coli*. Our previous studies have demonstrated that with the increase in temperature, *E. coli* cells lose their cultivability [23]. Overall, the present study may emphasize the revelation of stressed phenotypes of our laboratory strain which could be of interest to further unveil the fate of a single cell.

## Acknowledgements

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

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