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Physiological responses of *Escherichia coli* cells cultivated under a sublethal oxidative stress condition

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ABSTRACT

Superoxide dismutase (SOD) is widely distributed in organisms and alleviates toxicity of reactive oxygen species (ROS) formed inside cells. It was found that sublethal oxidative stress derived from photoexcited TiO_2 exerted a simulative effect on the growth of SOD null mutant of *Escherichia coli* (IM303) with reduction in intracellular ROS level. DNA microarray analysis was then carried out to compare gene expression between IM303 cells with and without the oxidative stress. From the DNA micro array data, *yfiD*, *yggB* and *yggE* were selected as genes up- regulated under the oxidative condition and then cloned into a pUC 19 plasmid. The original pUC 19 and constructed plasmids were introduced into *E. coli* MM294 and the transformants were cultivated in M9 medium with paraquat. Among these transformants, intracellular ROS content was the lowest in the cells carrying *yggE* gene and maximum specific growth rate of those cells was also higher than that of control cells with pUC 19. These results suggest that *yggE* gene product has an ROS-scavenging function in the cells of *E. coli* cells exposed to an oxidative stress and improves the efficiency of cellular growth.

Keywords: *Escherichia coli,* reactive oxidative stress, cell cultivation, photoexcited TiO_2.

INTRODUCTION

Oxidative stresses are generated by variety of mechanisms such as normal aerobic metabolism, exposure to ultraviolet light, metal ions, and oxidative components (Halliwell and Gutteridge, 1989, Canghai Lu *et al*., 2005, Stortz and Imlay, 1999, Pomposiello and Demple, 2000, Kren *et al*., 1988, Farr and Kogoma, 1991). The aerobic organisms have high reducing potential of molecular oxygen, and preferentially utilize oxygen for their vital function and proliferation (Inaoka *et al*., 1998). Consequently, reactive oxygen species (ROS) are often formed and can oxidize to damage various biomaterials owing to their high reactivity. Superoxide dismutase (SOD) is known as a key component in cellular defense against oxidative stress through reducing the intracellular concentration of superoxide anion to maintain cellular viability (Carlioz and Touati, 1986). It was demonstrated that E. *coli* mutants lacking in SOD displayed several defects in phenotypic features when the cells were exposed to an aerobic condition (Gort and Imlay, 1998).

During the last decade, important works report strong and fast photocatalytic cell inactivation of *E. coli* and different bacteriophages with TiO_2 (Ireland *et al.,*1993, Bekbolet, 1997, Armon *et al.,* 1998) and the plasmid DNA molecules were damaged by the oxidative stress in the TiO_2 photoreaction (Kim *et al.,* 2004a). It was reported that SOD-deficient mutants of *Escherichia coli* exhibited several defects, such as auxotrophy for amino acids, and high frequencies of spontaneous mutagenesis when grown aerobically (Gort and Imlay, 1998, Touati, 2002). These results also suggested that a sublethal or moderate level of oxidative stress may exert a triggering effect on activation of specific signaling, resulting in induction of altered metabolisms and cellular properties in SOD-deficient *E. coli* cells. However, recently the authors found that the population of SOD-efficient mutant of *E. coli* M303 contained a trace of spontaneously derived variant cells which prevailed during the culture under sublethal oxidative stress generated by photoexcited TiO_2 (Kim *et al*., 2004). This result suggested that the variant cells possibly acquired a certain defense system against the oxidative stress besides the SOD mechanism, and the isolates exhibited the outgrowing properties with increased specific growth values under both the conditions with, and without TiO_2 and light.

In the present study, the biological function of genes selected from the analysis was investigated in

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terms of cell growth and ROS content exposed to the oxidative stress generated by photoexicited TiO_2.

MATERIALS AND METHODS

Strain, media, cell growth, and cell isolation

A mutant of *E. coli*, SOD-deficient strain IM303 (I4), and its wild-type strain MM294 were used throughout the experiments. A modified M9 medium containing 8 g/L glucose was used with supplementation of amino acid mixture (2068 mixture, ATCC culture medium). IM303 (I4) cells were grown at 37 °C in an L-type test tube containing 10 mL of the medium with or without 10 mg/L TiO_2 particles (P25, Nippon Aerosil Co., Japan) under light irradiation at 12.5 W/m^2 with 20 W black light fluorescent lamps. In the case of MM294 culture, the cells were grown with 10 µmol/L paraquat (PQ) under the same condition in the absence of TiO_2 and light. Growth of the cells was monitored by measuring an optical density at 660 nm (OD_{660}). Maximum specific growth rate, μ_m, was estimated by fitting the obtained growth-curve data to the modified Gompertz equation (Zwietering *et al.,* 1990). To investigate effects of the selected gene on growth property, a bioreactor with 3 L vessel equipped with dissolved oxygen concentration and pH sensors (BMS-03PI, ABLE & Biott Co., Ltd., Japan) was used. Cultures of recombinant IM303 (I4) cells were conducted in the following condition: 1.0 L of modified M9 medium containing 50 µg/mL ampicillin and 10 µmol/L isopropyl-β–D-thiogalactopyran oside, 1 % inoculation, 1.0 L/min (1.0 v/v/m) aeration, and 500 rpm of agitation. Amount of cells was measured by monitoring OD_{660}, and converted into dry cell weight. Glucose concentration in the medium was measured (Kim *et al.,* 2005) by using an analytical instrument (BioProfile 200 Yamato Scientific Co., Ltd., Japan).

Irradiation experimental setup

The experimental setup is shown schematically in Figure 1. All the experiments were carried out in L-type test tube containing 10 mL of medium with or without 0.01 g/dm^3 TiO_2 particles (Degussa P25, Japan Aerosil Co., Japan) under shaking condition of 45 strokes per minute. The test tubes were irradiated from the upper side with a bank of 20 W black light fluorescent lamps with effective wavelengths of 300–420 nm (FL-20S BL-B, Matsushita Electric Industrial Co., Japan). The incident light intensity was adjusted by changing the vertical distance between the lamps and test tubes, and was recorded as an average measurements conducted on the tube surfaces with a quantum sensor (Model No. J-221, UVP Inc., USA).

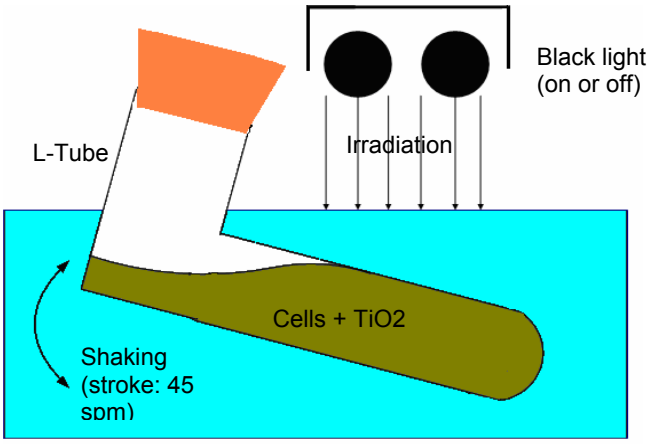

Figure 1: Experimental setup for cultivating *E.coli* strain under oxidative condition

DNA micro array analysis and construction of plasmid carrying a selected gene

IM303 (I4) cells cultivated with/without light-irradiated TiO_2 were harvested from the culture solution, and total RNA was extracted from the whole cells by employing a RNeasy Mini Kit and an RNase-free DNase Set (QIAGEN Inc., USA). Labeled cDNAs were prepared using a RNA Fluorescence Labeling Core Kit (Takara Bio Inc., Japan) with dUTP-Cy3 or dUTP-Cy5 fluorescent nucleotide. Assay of DNA microarray was conducted with an IntelliGene® *E. coli* CHIP (Takara Bio Inc., Japan). The microarray profiling data were compared between RNA samples from IM303 (I4) cultures with, and without the oxidative stress was generated by photoexcited TiO_2. The constitutive regions of the selected genes were amplified by PCR. A pUC 19 vector was digested with appropriate restriction enzymes, ligated with the amplified DNA fragments, and then applied for transforming *E. coli* DH5α. The plasmid DNAs multiplied in the cells were extracted, and purified.

Intracellular ROS content

IM303 (I4) and MM294 cells carrying pUC 19 vector or constructed plasmid were cultivated under indicated conditions, and an aliquot of culture broth was withdrawn from the test tube to determine the intracellular ROS content at OD_{660} = 0.5 and OD_{660} = 0.2. ROS content was quantified with 5-(and-6)-chloromethyl-2',7'-dichlorofluorescin diacetate (C-6827, Molecular Probes Inc., USA), and was expressed on a H_2O_2 equivalent basis (Krishnaiah *et al.,* 2006).

RESULTS AND DISCUSSION

The original population of SOD null mutant of *E. coli*, IM303, contained a trace of spontaneously derived variant cells, and these variant cells were permitted to survive, and prevail during the culture with the sublethal oxidative stress generated from light-irradiated TiO_2. The growth profiles of the culture of a typically selected isolate, IM303 (I4), with TiO_2 and light, and without TiO_2 and light were plotted as shown in Figure 2. The μ_m and t_L values were calculated with Gompertz equation as shown in Table 1. It is evident that the μ_m value with TiO_2 and light is approximately 2 times higher than that of the cells cultivated in the absence of TiO_2 and light whereas the lag time lengths were appreciably distinct. To investigate the genomic-wide expression profiles in IM303 (I4) cells cultivated under the oxidative stress, while comparing with those under the normal culture condition, DNA microarray analysis was performed. In total, 25 genes were up-regulated at a significant expression level by the oxidative stress and three genes, *yfiD*, *yggB* and *yggE*, were selected for further research because literatures dealing with these genes were very few and the gene products may function to protect bacterial cells from oxidative stress. Based on the results mentioned above, three plasmids, pYFD, pYGB and pYGE, containing *yfiD*, *yggB* and *yggE*, respectively, were constructed, and transformed into *E. coli* cells. Antioxidant functions of the genes were investigated in the cultures of the transformants under various oxidative conditions.

Table 1: Maximum specific growth rate μ_m (h^{-1}) and lag time t_L (h) under various conditions

Condition	μ_m	t_L
Without TiO_2 and light	0.20	7.8
With TiO_2 and light	0.39	19.7

The standard deviation was less than 0.01 per h

In general, cell growth rate can be linked to an intracellular ROS level, which may be fluctuated in response to oxidative stress against cells, although several antioxidant systems exist in living cells to avoid cellular damages caused by oxidative stress (Storz and imlay, 1999, Kim *et al*., 2004a). As shown in Figure 3, the ROS contents in yfiD, yggB and yggE are less than the control pUC19 at both pre-exponential OD levels (OD_{660} = 0.5 and OD_{660} = 0.2), despite ROS level in the transformant carrying *yggE* gene was suppressed significantly compared to *yfiD* and *yggB*, and further researches were focused on elucidating the functions of *yggE* gene product.

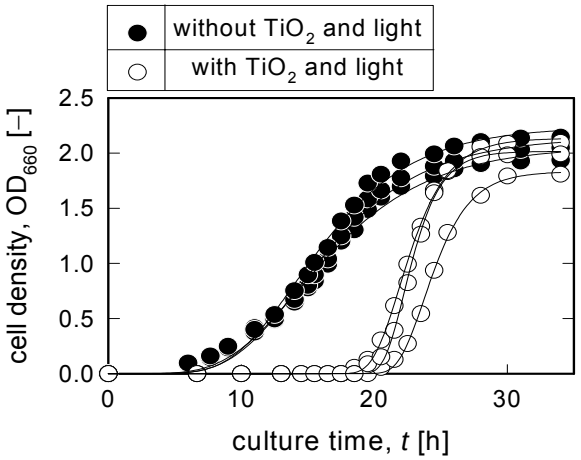

Figure 2: Growth profiles of typical isolate, IM303 (I4) cells grown in the modified M9 medium with and without TiO_2 and light. The solid lines were drawn by fitting the data to the modified Gompertz equation

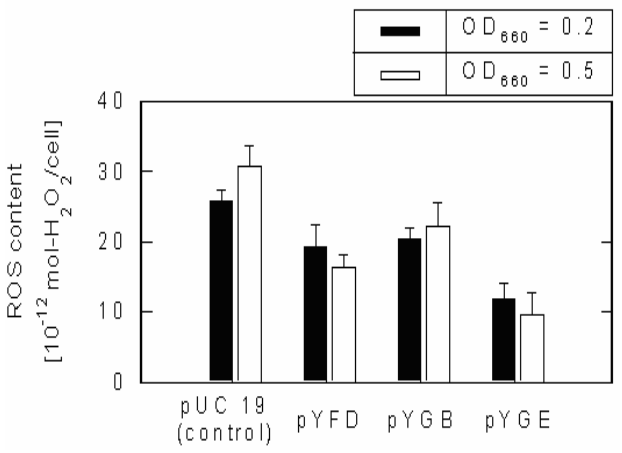

Figure 3: ROS contents in SOD-deficient mutant of *E. coli*, IM303 (I4) cells, carrying pUC 19, pYFD, pYGB and pYGE, measured at OD_{660} = 0.2 and 0.5. The cells were grown in the modified M9 medium without TiO_2 and light

Transformant carrying pYGE showed much higher μ_m value than that of control cells carrying pUC 19 (Duduku *et al*., 2006). For investigating the biological function of *yggE* gene under different stress conditions,

MM294 cells transformed by pYGE was cultivated in the medium with 10 µmol/L PQ. As shown in Figure 4(A), the μ_m value of the transformant was about 5 times higher than that of the control cells. In the case of ROS contents in the transformants carrying pUC 19 and pYGE, the latter cells showed significantly lower ROS levels than that of its counterpart as shown in Figure 4(B) at OD_{660} = 0.5 and OD_{660} = 0.2.

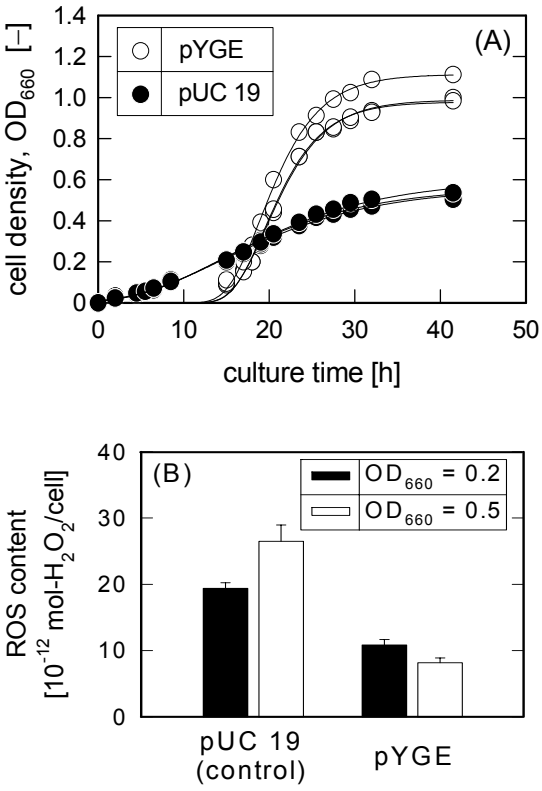

Figure 4: Growth profiles and ROS content in wild-type *E. coli* cells carrying pUC 19 and pYGE cultivated with 10 µmol/L PQ. **(A)** Growth profiles of MM294 cells. The solid lines were drawn by fitting the data to the modified Gompertz equation. **(B)** ROS contents in MM294 cells

To investigate the effect of *yggE* gene product on culture property, cultivation of *E. coli* IM303 (I4) carrying the plasmid pYGE or pUC 19 in a bioreactor was performed under aerobic conditions (Duduku *et al*., 2006). During the cultivation, DO concentration was kept at the value ranging from 6.0 to 6.8 ppm as shown in Figure 5(A), which seems to be sufficient to provide stress to the SOD-deficient cells, and the sensitivity of oxygen was reported (Semchyshyn *et al*., 2005). Figure 5(B) shows the growth profiles of *E. coli* IM303 (I4) cells carrying pYGE and pUC 19, and time profiles of glucose concentration in medium. The cell density remained unchanged for both the transformants. DO concentration for pYGE was found to be higher than the pUC19 while the glucose concentration of pUC 19 is higher than pYGE. IM303 (I4) cells carrying pYGE were remarkably enhanced in their growth activity when compared with that of the control cells carrying pUC 19. Furthermore, the dry cell weight obtained at the stationary phase of IM303 (I4) cells carrying pYGE was significantly higher than that of the control cells carrying pUC19 in spite of no significant difference in glucose consumption.

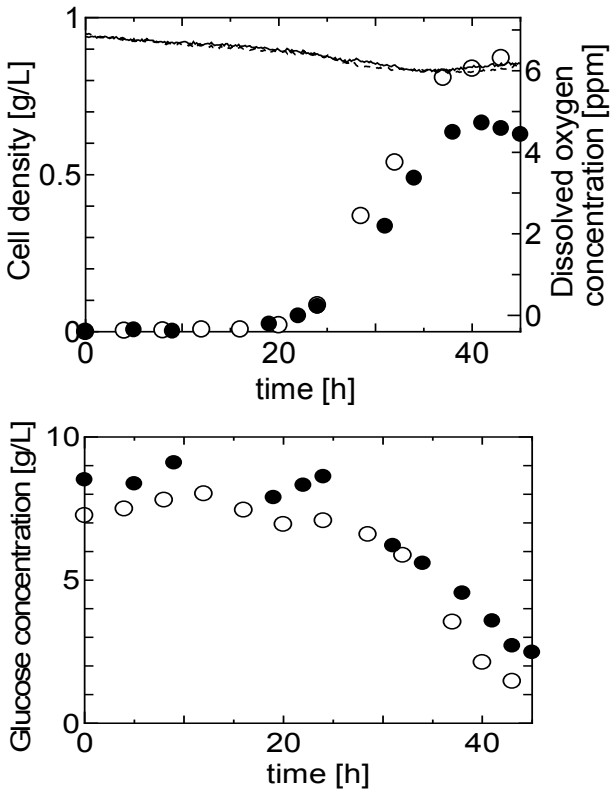

Figure 5: (A) Growth and dissolved oxygen concentration profiles of IM 303 (I4) cells carrying pYGE and pUC 19. **(B)** Time profiles of glucose concentration in the medium

CONCLUSIONS

Total gene expression profile in SOD-deficient *E. coli* cells was analyzed to understand their genotypic

features. Three genes were selected for investigating their antioxidant functions. ROS level in the transformant carrying *yggE* gene was suppressed significantly compared to yfiD and yggB under the photoexcited TiO_2 oxidative stress. IM303 (I4) cells carrying pYGE were remarkably enhanced in their growth activity when compared with that of the control cells carrying pUC 19. Outcomes of the present work will make notable contribution to antioxidant defense systems in the fields of environmental and industrial approaches.

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