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2015/05/06

Manuscript: **Characterization of two alkyl hydroperoxide reductase C (AhpC) homologs, AhpC_H1 and AhpC_H2 existed in *Bacillus subtilis***

Your reference # as an invited paper: 02500736

ESPS Manuscript NO: 16905

Dear Editor of WJBC

Thank you for your letter regarding the revision of our manuscript.

The revised manuscript entitled “**Characterization of two alkyl hydroperoxide reductase C (AhpC) homologs, AhpC_H1 and AhpC_H2 existed in *Bacillus subtilis***” by Mee-Kyung Cha, Yoo-Jeen Bae, Kyu-Jeong Kim, Byung-Joon Park, and myself is sending via electronic submission for publication in the original research article section of WJBC. Please note that three students (Yoo-Jeen Bae, Kyu-Jeong Kim, Byung-Joon Park) who supported the experiments throughout this research were added as the authors. All the authors have read and approved the revised manuscript and agree with its submission to your esteemed journal. There are no conflicts of interest to declare.

This manuscript has been revised along with lines as suggested. Please note that highlight the changes to our manuscript within the document by using **red colored text** except for the correction of errors in grammar and spelling. The manuscript has been examined and extensively revised in English by an English-language native scientist (see the attached “Certificate of English Editing”).

Specific responses follow:

Reviewer (02254242)’s Comment:

Specific comments

1. Page 17/Figure 10. Do the investigators have an estimation of the increase in AhpC H1 in response to the different types of oxidative stress? There should be a way to estimate the increased production.
2. Figure 11. The authors should briefly explain the growth curves in aerobic culture in. There is a decrease in cell density indicating that the cell numbers are decreasing for the mutants. Were the cells first cultured anaerobically? That type of detail should be in the materials and methods or results section.
3. Figure legends should be clearly labeled. For example the contents of Figure 2A1 are not clearly under 2A1. The labels should be 2A, 2B, 2C, etc. and not have the subheadings.

Response 1: We have added the text concerning issue as follows: “**Western blotting analysis indicates that AhpC_H1 levels were not significantly affected by H₂O₂, paraquat, and diamide; however, organic peroxides, especially CMOOH, markedly increased AhpC_H1 expression in *B. subtilis*, suggesting the function of AhpC_H1 as an antioxidant scavenger of lipid hydroperoxides.**”

Response 2: The seed culture condition has added in the legend of Fig. 11 as follows: “(A) Bacterial growth was monitored by the optical density at 600 nm (OD₆₀₀) for up to 25 h **after addition of the same cell density of exponentially grown culture in aerobic condition;**”

Response 3: Figure 2 legend has been clearly labeled as suggested. Please find the correction in corresponding **text** and the **legend**.

Reviewer (00502963)’s Comment:

Specific comments:

1. In cladogram of figure 1, the bootstrap values should be shown at each branch.
2. Were these recombinant proteins produced in *E. coli* purified under nature conditions or denatured conditions?
3. A statistical analysis should be done in figure 8.

Response 1:

We have added following sentence in Material & Method section and the revised Figure 1. “Phylogenetic and molecular evolutionary analyses were conducted using MEGA (Molecular Evolutionary Genetics Analysis) version 6. A phylogenetic tree was generated by using the Maximum Likelihood method based on the JTT matrix-based model. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site (next to the branches).”

Response 2:

We have added following sentence in Material & Method section. “The soluble recombinant proteins were produced in *E. coli*.”

Response 3:

The statistical analysis has been carried out. Please see the revised Fig. 8.

Please address all correspondence to me at the above address. We anticipate a fair and expedient review. Thank you in advance for all of yours

Sincerely yours,

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Please address all correspondence to me at the above address. We anticipate a fair and expedient review. Thank you in advance for all of yours

Sincerely yours,

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