

Response to Reviewer 1 Comments

Basically, this manuscript provides some fundamental data with novelty. However, there are some concerns needed to be addressed before publication.

Point 1: In Figure 3, I recommend the authors to combine Figure 3A and Figure 3B to on graph.

Response 1: We appreciate very much for the Reviewer's good comments and kind recommendation. This part has been combined in this version, please see page 6 Figure 3.

Point 2: In Figure 3B lane 4, the band for rHsGST is not obvious. In addition, the authors did not explain the molecular weight and purity of rHsGST in the text.

Response2: We appreciate very much for the Reviewer's comment. This part was revised, please see page 5 line 129-132 and page 6 Figure 3.

Point 3: The authors did not mention how to refold rHsGST.

Response 3: We appreciate very much for the Reviewer's comment. This part was added, please see page 10 line 244-247.

Point 4: In lines 163-164, this sentence seems not related to the topic discussed.

Response 4: We appreciate very much for the Reviewer's useful suggestion. This part has been deleted.

Point 5: In Figure 6, the authors overexpressed HsGST in BL21. Generally, the overexpressed proteins within *E. coli* are in the form of inclusion bodies and do not refold properly. I wonder how the overexpressed HsGST exhibits its biological functions. The authors should explain this point.

Response 5: We appreciate very much for the Reviewer's good comments and kind recommendation. It's normally that the overexpressed proteins within *E.coli* are in the form of inclusion bodies. In this study, the treatment of the inclusion bodies of HsGST

were added (please see page 10 line 244-247), and the enzyme activity of the purified rHsGST were also detected. This method has been widely used in the studies of our laboratory [Wang, Y. et al, *Marine Drugs* 2018 16(10); Wang, Y. et al, *Int. J. Biol. Macromol.* 2018] and other reported studies [Sandamalika, W.M.G et al, *Fish Shellfish Immun.* 2018, 77, 252-263; Zhang, Y.Y. et al, *Cell Stress Chaperon.* 2013, 18, 503-51.].

Point 6: In Figure 6, I think the control of pET-28a(+)-unrelated protein (for example BSA or others) is lacking.

Response 6: In this study, BL21/pET-28(+) was set as the control, which accorded with the principle of single factor experiment design and could explain the functions of HsGST.

Point 7: In Figure 6, the authors did not mention the concentration of hydrogen peroxide used.

Response 7: We appreciate very much for the Reviewer's comment. This part was revised, please see page 11 line 280.

Point 8: In Figure 6, can the authors compare the oxidative stress-protective effects of HsGST with other GSTs?

Response 8: We highly agree and appreciate very much for the Reviewer's nice comments. The comparisons of the GSTs have been revised, please see page 9 line 206-207.

Point 9: In line 208, the authors should mention who isolated the strain ANT108.

Response 9: We appreciate very much for the Reviewer's useful suggestion. This sentence was revised in this version, please see page 9 line 220-221.

Point 10: In line 272, the authors have mentioned that rHsGST may have the potential application as functional foods and therapeutic agents. However, there are already numerous GSTs available in the market. The authors need to specify this point.

Response 10: We appreciate very much for the Reviewer's good comments and kind recommendation. The potential application of rHsGST has been added, please see page 11 line 289-291.