

## Answer letter

Dear Boudewijn de Jonge,

We have modified according to the comments of the reviews. The modification traces can be checked in the manuscript. We have answered these questions as follows. Please do not hesitate to contact me if you have any questions, Thank you.

### Reviewer #1

Major revision

1、 Authors should include details about the combination of Lla-Met, such as the formulation ratio and instructions on how to make it.

Answer:

(1)The ratio of linolenic acid and metronidazole is 1:1.

(2)Lla-Met was prepared with specific reference to the manuscript of Linolenic Acid-Metronidazole: a Compound Relieving Drug Resistance and Inhibiting *Helicobacter pylori*.

**Answer:** We have provided details about the combination of Lla-Met, please check the traces in manuscript.

2、 How many replicates were used for each experiment?

**Answer:**We have revised, three biological repeats were performed for each experiment,please check the trace.

### Reviewer #2

Minor suggestions

1、 Although metonidazole is a relatively cheap ...

**Answer:** We have revised this sentence to “Although metonidazole is a widely used and cost-effective”.Please check the traces in manuscript.

2. Please modify the sentences: "Therefore, Lla-Met could be used for preparing antibiotics. It is ideal. But the antibacterial mechanism of the Lla-Met compound remains unclear, this study explored this mechanism", as currently it is difficult to understand the meaning.

**Answer:**We have revised this sentence to "Therefore, Lla-Met would serve as promising antibiotics. However, its antibacterial mechanism is poorly understood, this study explored this mechanism".Please check the traces in manuscript.

3.Section 1.3. please provide the model of microscope used here.

**Answer:**We have provided the model of microscope used.Please check the traces in manuscript.

4.Please modify the sentence: "a final concentration of 10 µg/mL PI at 37°C protected from the light 30 min", as currently it is difficult to understand the meaning.

**Answer:**We have revised this sentence to "a propidium iodide solution (PI,10 µg/mL,Thermo Fisher) at 37 °C protected from light 30 min".Please check the traces in manuscript.

5.Please modify the sentence: "Lla-Met induced the expression of ROS, MdaB, and SodB, all anti-oxidation-related genes in Table 2".

**Answer:**We have revised this sentence to "Lla-Met is inducing production of ROS in *H. pylori*, and therefore an increased expression of MdaB and SodB, both of which are associated with protection against the oxidative stress".Please check the traces in manuscript.

6.Please modify the sentence: "In this paper, the mechanism of linolenic-metronidazole compound was demonstrated to involve inhibiting *H.*

*pylori* growth by up-regulating superoxide dismutase MdaB and SodB genes, resulting in excessive ROS accumulation"

**Answer:**We have revised this sentence to "In this paper, the mechanism of linoleic-metronidazole compound was demonstrated to involve inhibiting *H. pylori* growth by inducing excessive ROS accumulation,resulting in excessive superoxide dismutase MdaB and SodB genes expression ". Please check the traces in manuscript.

7.Please modify the sentence: "The results showed that excessive accumulation ROS could affect viability of *H. pylori* by 20.5% ..."

**Answer:**We have revised this sentence to"The results showed that excessive accumulation of ROS could affect viability of *H. pylori* by reducing it to 20.5%... " Please check the traces in manuscript.

Sincerely,

Yan-qiang Huang

2023-7-14