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Inhibitory effects of D-ribose on biofilm development of *Escherichia coli*

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ABSTRACT

Inhibition of biofilm formation can promote the operation of water system such as membrane bioreactor. This paper investigated the effects of D-ribose on biofilm development of *Escherichia coli*. The results showed that the D-ribose could arrest biofilm formation of *E. coli*. The decrease in attached biofilm biomass reached 17.95% with 100 μM D-ribose. Bacterial attachment experiments indicated that the attachment of *E. coli* to polyvinylidene fluoride membrane was inhibited with D-ribose (10 μM to 5 mM). And the attachment was weak with the addition of D-ribose. Further research showed that 10 μM–5 mM D-ribose could inhibit the extracellular polymeric substance (EPS) secretion of *E. coli*. The inhibition was obvious at 100 and 500 μM D-ribose. Moreover, the addition of D-ribose increased superoxide dismutase and glutathione peroxidase activities, which might be the reason of EPS reduction.

Keywords: Biofilm; Extracellular polymeric substance (EPS); D-ribose; Bacterial attachment; Enzyme activity

1. Introduction

Biofilm, a major survive mode of bacteria, helps bacteria to survive under unfavorable conditions [1]. In water and wastewater treatment systems, biofilm can result in the damage of equipment surfaces, membrane fouling of membrane bioreactor, the corrosion of water pipelines, and contamination of the water transported [2,3]. Therefore, it is necessary to find an effective way to inhibit the formation of biofilm.

Recently, more and more researches have focused on the methods of biofilm suppression based on quorum-sensing quenching [4]. Quorum sensing is a method of cell-to-cell communication based on microbial population density [5,6]. Microbial population behavior is regulated by a class of small molecular organisms called signaling molecules. When the concentration of signaling molecules such as autoinducer-2 (AI-2) in the environment reaches a certain threshold, the special behavior of the microbial community (biofilm formation, toxin production, etc.) will be excited and expressed [7,8]. Based on this principle, quorum sensing inhibitors have been proposed and recognized as a sensible method to inhibit the formation of bacterial biofilm [9]. It can inhibit the formation of biofilm by controlling the amount of signal molecules using quorum sensing inhibitors.

The ideal quorum sensing inhibitors should have a significant interference effect on the bacterial population and be non-toxic to the target [10]. It is suggested that the activity of AI-2 is inhibited by the addition of ribose, which inhibits the formation of biofilm. Since ribose and AI-2 have similar chemical structure, there might be competition relationship between them. Other studies have shown that ribose stimulates or inhibits the expression of genes in glycolytic pathway to inhibit biofilm formation [11]. In addition, Armbruster et al. [12] suggested that ribose lead to the defects in biofilm maturation. Lee et al. [13] indicated that ribose significantly inhibited the biofilm formation of *Streptococcus* and the expression of related genes. Recently, it has been found that D-ribose is non-toxic and has good quorum-sensing inhibition effect, which can be used as an inhibitor of

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biofilm formation. Liu et al. [14] considered D-ribose inhibited biofilm formation of *Lactobacillus paraplantarum* L-ZS9 by regulating genes expression of glycolytic process, extracellular DNA transcription and translation.

These studies indicated that ribose can inhibit the formation of bacterial biofilms such as *Lactobacillus paraplantarum* L-ZS9 and *Streptococcus*. However, the effect of D-ribose on bacterial extracellular polymeric substance (EPS) and enzyme activity has not been studied. EPS, which are considered to be important substance determining the properties of bacteria, directly affect the formation of biofilm. It would be interesting to know the effect of D-ribose on the characteristics of *Escherichia coli* such as EPS, adhesion and desorption, etc. Therefore, this paper takes *E. coli* as a model bacterium. The biofilms biomass of *E. coli* was measured at different concentrations of D-ribose. The adhesion and desorption of *E. coli* to the polyvinylidene fluoride (PVDF) membrane were analyzed. Production and properties of EPS by *E. coli* were examined. Antioxidant enzyme activities of *E. coli* were also compared to obtain more information. This paper provides a new perspective for the inhibition of D-ribose on biofilm formation by *E. coli*, and is valuable for potential application of D-ribose to control biofilm formation in water system.

2. Materials and methods

2.1. Chemicals

D-ribose (purity 99%) were purchased from Solarbio (Beijing Solarbio Science & Technology Co., Ltd., China). Inorganic salts (NaCl, KCl, KH_2PO_4 and K_2HPO_4) were bought from Zhiyuan Chemical Reagent Co., Ltd., (Tianjin, China). 96-well plates were bought from Corning (NY, United States). Distilled water was used in this study.

2.2. Bacterial strains and culture

E. coli was used as model strain in all experiments. Bacterials were cultured in Luria–Bertani (LB) medium [15] with 0 μM, 10 μM, 100 μM, 500 μM, 1 mM, 5 mM and 10 mM D-ribose, respectively. The culture was incubated at 37°C for 27 h with shaking at 110 rpm until the culture to a stable period [16].

2.3. Determination of EPS

EPS of *E. coli* was extracted using the heat extraction method [17]. *E. coli* suspension were centrifuged at 9,000 rpm for 5 min and re-suspended in distilled water, then heated at 80°C for 30 min. The mixed liquor was centrifuged at 9,000 rpm for 5 min. The 0.45 μm filter membrane was used for filtration. The filtrate was regarded as EPS. Protein and carbohydrates were determined by Folin-phenol method [18] and sulfuric acid-phenol method [19], respectively.

2.4. Bacterial attachment experiments

The adhesion substrate was PVDF flat-sheet membrane with a diameter of 47 mm and a pore size of 0.22 μm. Before use, PVDF membrane was soaked in 75% ethanol solution for 30 min, and washed by distilled water [20].

Bacterial samples were collected after 27 h incubation. The bacterial sample was re-suspended in 10 mM KCl, and OD_{600} of the suspension was adjusted to 0.4–0.5. Each beaker contained 25 mL bacterial suspension with or without D-ribose. Initial bacterial concentrations were determined by measuring OD_{600} (recorded as C_0). The membrane was placed in bacterial suspensions. The beakers were shaken for 2 h at 25°C, 50 rpm. After that, membrane samples were taken out carefully and the suspensions were determined by measuring OD_{600} again (recorded as C_e). Then the PVDF membrane was rinsed with another 25 mL 10 mM KCl. The flushing solution were determined by measuring OD_{600} (recorded as C'_e). Adhesion rate and desorption rate of *E. coli* were calculated by Eqs. (1) and (2):

$$A(\%) = \frac{C_0 - C_e}{C_0} \times 100\% \quad (1)$$

$$R(\%) = \frac{C'_e}{C_0 - C_e} \times 100\% \quad (2)$$

2.5. Biofilm formation assay

Biofilm formation of *E. coli* were measured by crystal violet (CV) staining [21]. Briefly, after 27 h incubation, *E. coli* with OD_{600} of 0.1 was dispersed in the medium. After dilution, it was placed in a 96-well sterile polystyrene cell culture plate and cultured without rocking at 37°C for 24 h. The bacteria liquid in the well plate was transferred and stained with 150 μL 1% CV for 15 min. Then the well was rinsed with PBS buffer for 3 times. 150 μL 95% ethanol solution was added into each well plate. The absorbance was determined at 590 nm using the Synergy 2 microplate reader (Biotek, Winooski, VT, United States).

2.6. Determination of antioxidant enzyme activity

30 mL bacterial suspension after cultured for 27 h was centrifuged at 9,000 rpm for 5 min. The supernatant was discarded and the precipitation was mixed with 0.9% saline to the original volume, which was recorded as bacterial solution I. 5 mL was taken from bacterial solution I and diluted to 50 mL with 0.9% saline, which was recorded as bacterial solution II. The bacterial solution II was broken by an ultrasonic cell disruptor with an ultrasonic frequency of 400 W and an ultrasonic interval of 2 s for 10 min [22,23]. The supernatant was treated as crude enzyme solution. According to Chang et al. [24], the activities of glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) were detected.

3. Results and discussion

3.1. Effects of D-ribose on biofilm formation

The effect of D-ribose concentration on biofilm formation of *E. coli* was investigated (Fig. 1).

After incubated for 27 h, the biofilm biomass of *E. coli* was significantly reduced in the presence of D-ribose (Fig. 1). With increasing concentration of D-ribose (10 to 100 μM), the biofilm biomass decreased gradually. Attached biofilm biomass decreased by about 17.95% at 100 μM D-ribose. As the concentration of D-ribose increased from 500 μM to 10 mM, the reduction rate of biofilm biomass decreased from 16.76% to 11.49%. This result suggested that the D-ribose could arrest biofilm formation of *E. coli*. This was in agreement with previous study that D-ribose could inhibit biofilm formation of *Lactobacillus paraplantarum* L-ZS9 [14]. However, Liu et al. [14] demonstrated that the inhibition of D-ribose on biofilm formation of *Lactobacillus paraplantarum* L-ZS9 increased significantly with increasing concentration of D-ribose. And there was non-monotonic correlation between D-ribose concentration and biofilm inhibition in this study. The inconsistency of these results might be due to the differences in the range of D-ribose concentrations (10 mM–100 mM in their study and 10 μM–10 mM in this study, respectively). D-ribose, which could also inhibit biofilm formation of *E. coli* in this study, might be used as a potential bacterial biofilm inhibitor.

3.2. Effects of D-ribose on bacterial attachment

Attachment is an important process of biofilm formation. Strong attachment is beneficial to the formation of biofilm. The effect of D-ribose on *E. coli* attachment and desorption to the PVDF membrane was studied. As shown in Fig. 2a, the adhesion rate of *E. coli* in control group was 8.14%. With D-ribose concentration increased from 10 to 500 μM, the adhesion rates of *E. coli* decreased from 6.63% to 6.17%. When D-ribose concentration increased from 1 to 10 mM, the adhesion rates of *E. coli* increased from 7.59% to 8.89%. These results suggested that the presence of D-ribose could inhibit the initial adhesion of *E. coli* at low (10, 100, 500 μM and 1 mM) concentrations. The inhibitory effect is most obvious at the concentration of 500 μM. At high concentrations (5 and 10 mM), the presence of D-ribose led to increment (6.14% and 9.21%) in *E. coli* biofilm.

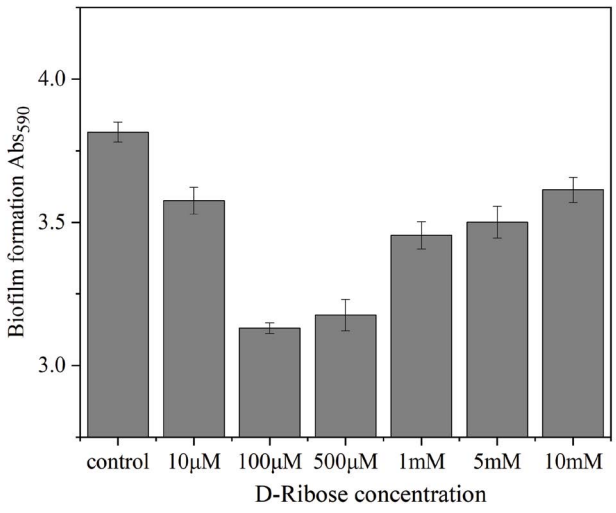

Fig. 1. Effects of D-ribose on biofilm formation of *E. coli*.

The effect of D-ribose on *E. coli* desorption to the PVDF membrane is shown in Fig. 2b. Desorption rate of *E. coli* in control group was 30.59%. All D-ribose concentrations tested significantly increased desorption rates (53.28%–65.32%) of *E. coli*, with the maximum impact observed at 100 μM D-ribose. This suggested that the attachment of *E. coli* to PVDF membrane was weak and the attached bacteria shed from attached membrane more easily with D-ribose treatment. In other words, the addition of D-ribose reduced the irreversible adhesion of *E. coli*.

Yu et al. [25] found that the addition of D-tyrosine could inhibit biofilm formation of *Pseudomonas aeruginosa* on nanofiltration membrane by reducing *Pseudomonas aeruginosa* attachment. Similar results were obtained using D-ribose as an additive in this study. The effect of D-ribose concentration presented a non-monotonic pattern. This suggested that the dosage of D-ribose used to inhibit biofilm formation should be carefully controlled.

3.3. Effects of D-ribose on EPS production and properties

Characteristics of EPS play a decisive role for biofilm formation [14]. Fig. 3 shows the EPS in *E. coli* with the effect of D-ribose.

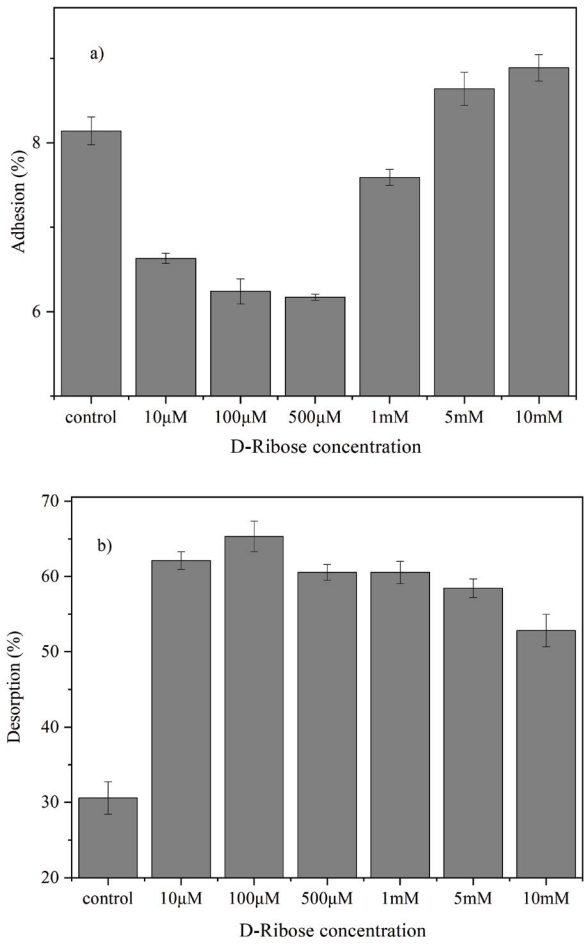

Fig. 2. Effects of D-ribose on (a) adhesion and (b) desorption of *E. coli*.

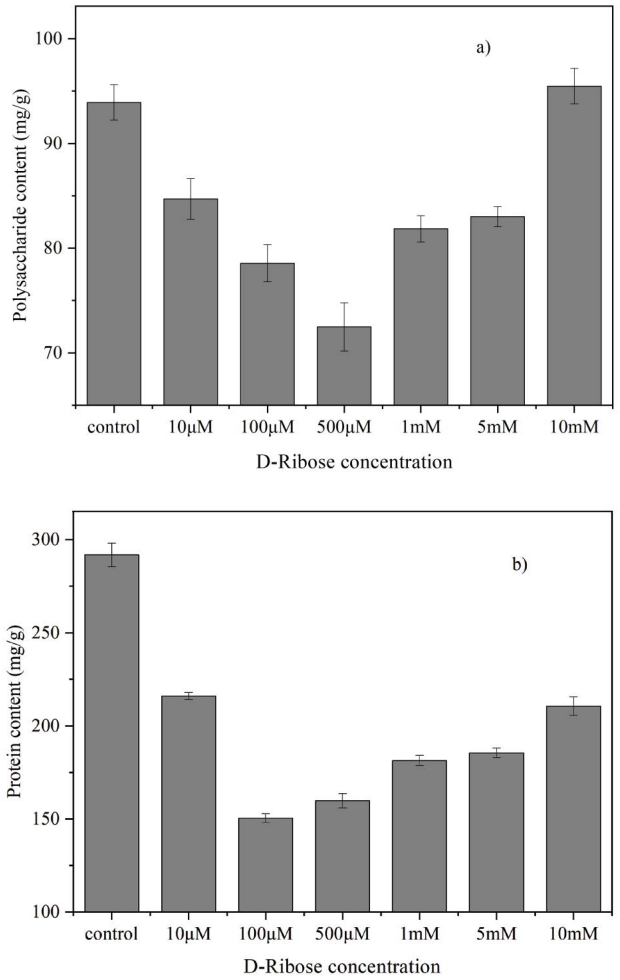

Fig. 3. Effects of D-ribose on EPS production of *E. coli* (a) carbohydrates and (b) protein.

As shown in Fig. 3a, compared to controls, the concentrations of carbohydrates in EPS were decreased gradually from 93.92 to 72.48 mg/g with increasing concentrations of D-ribose (10 to 500 μM). With further increase in D-ribose concentration, the carbohydrates content in EPS increased gradually up to 95.47 mg/g. With the increase in D-ribose concentration, the protein content in EPS decreased firstly and then increased. When the concentration of D-ribose was 100 μM, the protein concentration in EPS was 150.39 mg/g, showing the most obvious inhibition effect.

EPS, which is the main component of bacterial biofilm, is the bridge between cell and substrate surface. With the effect of EPS, the bacteria can adhere to the substrate surface and accumulate gradually to form biofilm. Liu et al. [14] deduced that D-ribose could regulate EPS synthesis of *Lactobacillus paraplantarum* L-ZS9. The results obtained in this study confirmed that the addition of D-ribose inhibited EPS production of *E. coli*. Moreover, D-ribose at 100 and 500 μM showed obvious inhibitory effect on EPS of *E. coli*.

The Excitation–emission matrix (EEM) fluorescence characteristics of EPS were further investigated to obtain more information. As shown in Fig. 4, there were three peaks, with E_x/E_m at 280/312~314 nm (tyrosine-like protein), 285/351~357 nm (tryptophan-like protein) and 325~350/395~402 nm (humic-like acids), respectively [26,27]. The peak intensities are shown in Table 1. Compared to control, the addition of D-ribose did not change the composition of EPS, but weakened all three peaks in EPS. When D-ribose was 100 μM, the maximum fluorescence values of tyrosine-like protein, tryptophan-like protein and humic-like acids were 550.4, 651.9 and 412.1, respectively, indicating the concentrations of tyrosine-like protein, tryptophan-like protein and humic-like acids in EPS were the lowest under this condition. This was consistent with the determination of protein content in *E. coli* EPS.

The size distribution of *E. coli* EPS was also studied (Fig. 5). The average size and polymer dispersion index (PDI) of EPS in the control group were 129.1 nm and 0.092, respectively, with a single peak near 100 nm. The average size and PDI of EPS were 178.9 nm and 0.471 with 10 μM D-ribose, 195.7 nm and 0.554 with 100 μM D-ribose and 256.4 nm and 0.59 with 500 μM D-ribose, respectively. For these concentrations, the peak value at 100 nm decreased and two new peaks at 1,000 and 5,500 nm appeared. The reason may be that D-ribose at 10 to 500 μM caused the aggregation of small molecular proteins in EPS, which led to the appearance of larger particle size agglomerates. When the concentrations of D-ribose were more than 1 mM, EPS showed a single peak, and the peak location was similar to that of control group.

These results suggested that D-ribose could inhibit EPS secretion of *E. coli* and changed some characteristics of EPS. The changes of these properties may lead to the inhibition of biofilm formation.

3.4. Effects of D-ribose on antioxidant enzyme activity

Effect of D-ribose on Antioxidant enzyme activities of *E. coli* are shown in Fig. 6. Compared with the control, D-ribose increased the SOD enzyme activity of *E. coli*. The SOD enzyme activity with 500 μM D-ribose was higher than the other groups (Fig. 6a). When the D-ribose concentration were 100 μM and 1 mM, the promotion effects on SOD enzyme activity were also quite remarkable. The GSH-Px enzyme activity (Fig. 6b) showed that the addition of D-ribose increased GSH-Px enzyme activities, and D-ribose at 100 μM exhibited higher promotion effect than other groups.

These results revealed that both the SOD and GSH-Px enzyme activities were greatly promoted by D-ribose, which was probably attributable to the regulation of multiple genes including glycolysis and other biological sugar metabolic and genetic processes [14]. The addition of D-ribose might promote the expression of enzyme-related genes, increase the activity of antioxidant enzymes, and lead to the reduction of peroxide in microorganisms and the decrease in EPS secretion. The mechanisms by which D-ribose affects the enzyme activities and EPS secretion of *E. coli* need to be further clarified by detailed analyses.

4. Conclusion

D-ribose could inhibit biofilm development of *E. coli* and there was non-monotonic correlation between D-ribose

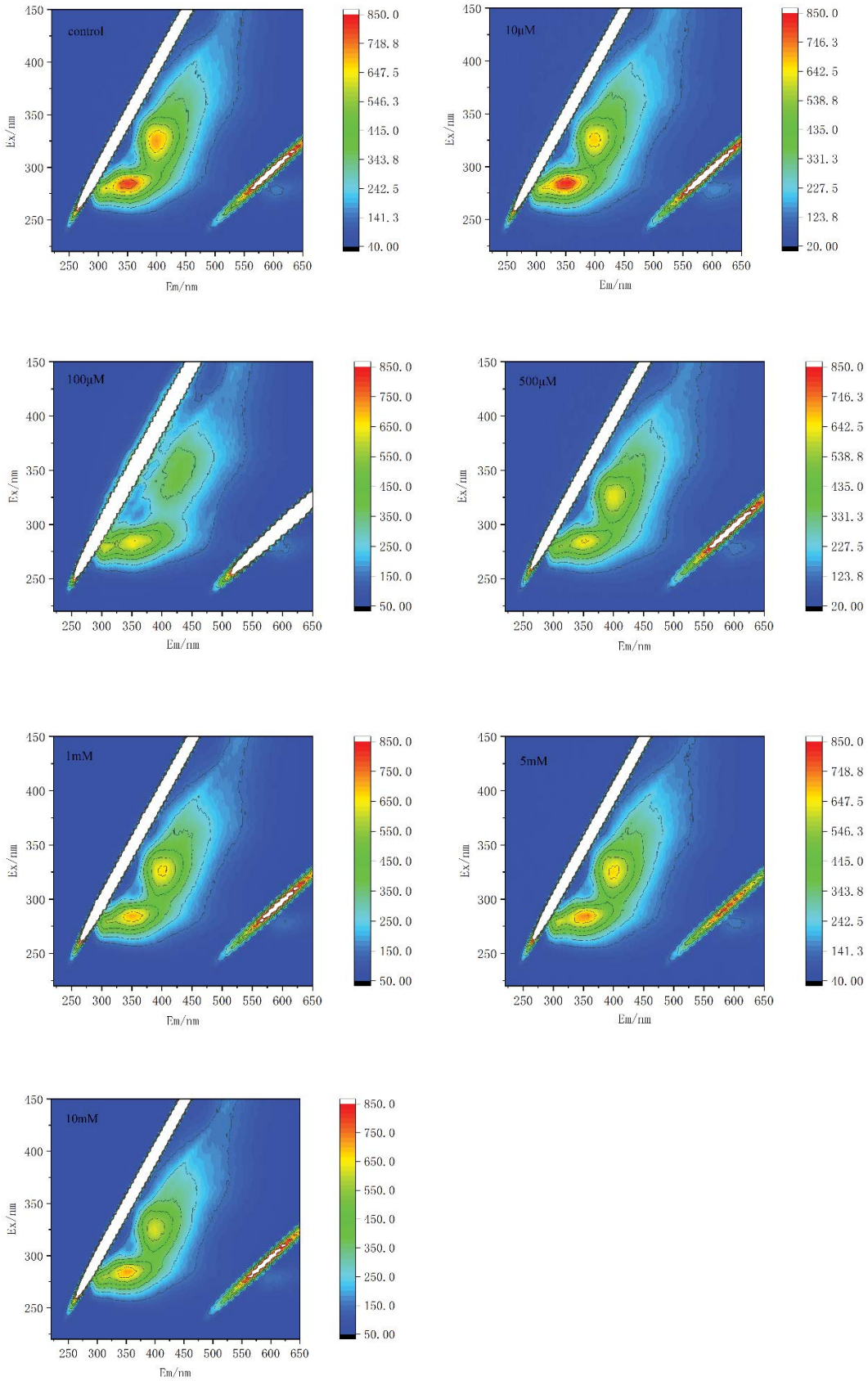

Fig. 4. Effect of D-ribose on EEMs of *E. coli* EPS.

Table 1
Fluorophore characteristics of EPS

Substance	Tyrosine-like protein		Tryptophan-like protein		Humic-like acid	
	E_x/E_m/nm	Peak	E_x/E_m/nm	Peak	E_x/E_m/nm	Peak
Control	280/313	664.4	285/351	817.7	325/399	721.6
10 μM	280/313	663.9	285/356	816.6	325/402	683.9
100 μM	280/314	550.4	285/355	651.9	350/428	412.1
500 μM	280/313	565.8	285/353	669.9	325/395	630.4
1 mM	280/314	576.4	285/358	720.5	325/402	677.3
5 mM	280/312	622.7	285/357	750.2	325/399	680.7
10 mM	280/313	668.9	285/356	836.6	325/402	683.9

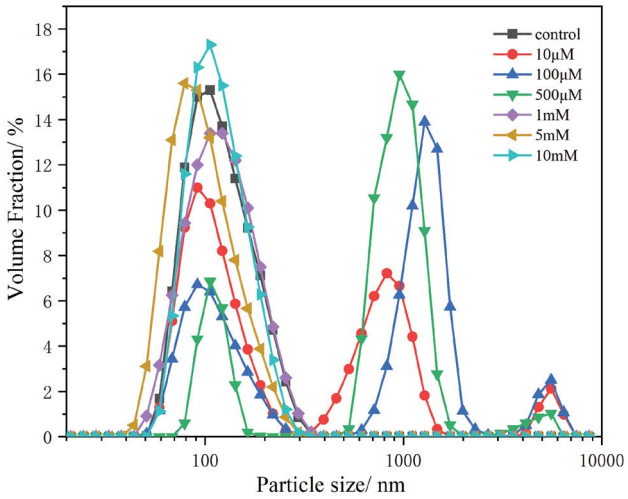

Fig. 5. Effect of D-ribose on particle size of *E. coli* EPS.

concentration and biofilm inhibition. The decrease in attached biofilm biomass reached 17.95% with 100 μM D-ribose. D-ribose reduced the adhesion of *E. coli*, and inhibited the secretion of EPS. Furthermore, SOD and GSH-Px activities were promoted greatly, which might be the reason of EPS reduction.

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Declarations

Conflict of interest: The authors declare that they have no conflict of interest.

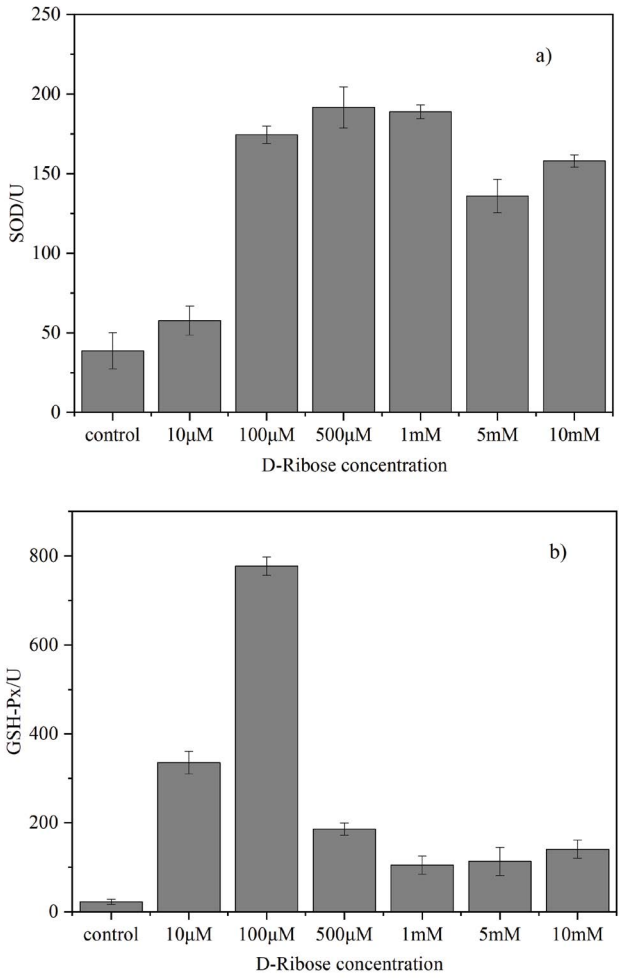

Fig. 6. Effect of D-ribose on enzyme activities of *E. coli*.

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