Electronic Supplementary Material (ESI) for RSC Advances.

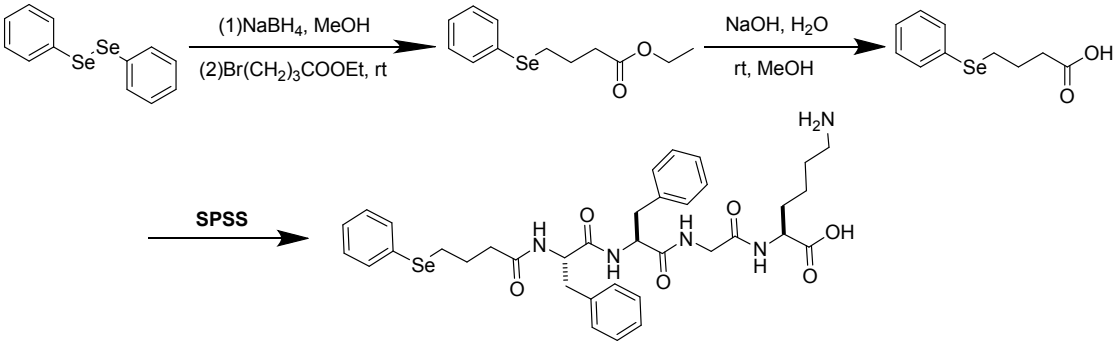
Supporting Information

Materials and methods:

Chemicals: Fmoc-amino acids were obtained from GL Biochem (Shanghai). Diphenyl diselenide, sodium borohydride, ethyl 4-bromobutyrate, 4-nitrophenol acetate, and ascorbic acid (vitamin C) were purchased from Aladdin Chemistry CO. Ltd. Commercially available reagents were used without further purification, unless noted otherwise. Nanopure water was used for all experiments. All other chemicals were reagent grade or better.

General methods: The synthesized compounds were characterized using ^{1}H NMR (Bruker ARX 400). ESI-MS spectrometric analyses were performed at the Thermo Finnigan LCQ AD System. HPLC was conducted at LUMTECH HPLC (Germany) system using a C_{18} RP column with MeOH (0.1% of TFA) and water (0.1% of TFA) as the eluents. TEM images were done on a Tecnai G2 F20 system, operating at 200 kV, TEM samples were prepared as following: a carbon-coated copper grid (from Zhongjingkeyi Technology Co. Ltd., Beijing, P. R. China) was vertically dipped into the solutions for 5 seconds and then placed in a desicator overnight before the TEM measurement. LC-MS was recorded on the LCMS-2020 (Shimadzu) system.

Synthesis and characterizations:



Scheme S-1. Synthesis of compound ***1***

Synthesis of ethyl 4-(phenylselanyl)butanoate: To a solution of diphenyldiselane (312 mg, 1 mmol) in MeOH (10 mL) under N_2, 2 mL of $NaBH_4$ (152 mg, 4 mmol) solution was injected. The ethyl 4-bromobutanoate (427 mg, 2.2 mmol) was then added. The mixture was stirred at room temperature over night. The solvent was removed by vacuum; 40 mL of water was then added. The aqueous mixture was extracted with EA (30 mL × 3), the organic layer was then combined and concentrated to give 500 mg of crude ethyl 4-(phenylselanyl)butanoate, which was directly reacted for next step without purification.

Synthesis of 4-(phenylselanyl)butanoic acid: To a solution of 4-(phenylselanyl)butanoate (500 mg, crude) in 20 mL of MeOH, KOH (224 mg, 4 mmol) in 2 mL of water was added. The mixture was stirred at room temperature over night. The solvent was removed by vacuum; 40 mL of water was then added. The pH value was adjusted to 13 by 4 mol/L KOH solution. The organic mixture was extracted by PE (20 mL × 3) and Et_2O (20 mL × 2), respectively. The pH value of the water layer was then adjusted to 2, and then it was extracted with EA (20 mL×3). The organic layer was concentrated to give 4-(phenylselanyl)butanoic acid (400 mg) with good purity and total yield was 41%.

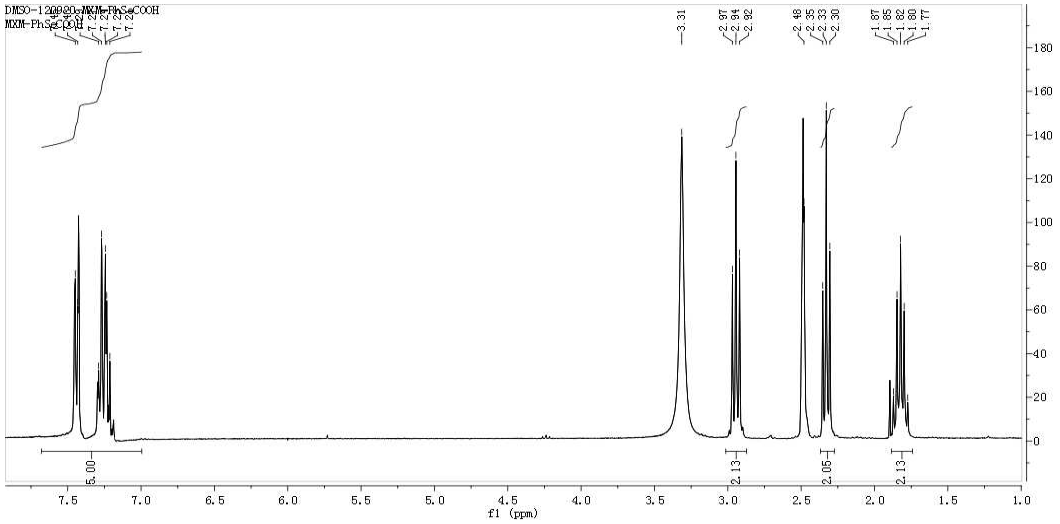


Figure S-1. ^{1}H NMR of *4-(phenylselanyl)butanoic acid*

Synthesis of $PhSe(CH_2)_3COFFGK$: The $PhSe(CH_2)_3COFFGK$ was synthesized by solid phase peptide synthesis (SPPS) using 2-chlorotrityl chloride resin and the corresponding N-Fmoc protected amino acids with side chains properly protected bydifferent group. The first amino acid was loaded on the resin at the C-terminal with the loading efficiency about 1.0 mmol/g. 20% of piperidine in anhydrous N,N'-dimethylformamide (DMF) was used to deprotect the Fmoc group. Then the next Fmoc-protected amino acid was coupled to the free amino group using O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluroniumhexafluorophosphate (HBTU) as the coupling reagent. The growth of the peptide chain was according to the established Fmoc SPPS protocol. At the final step, 4-(phenylselanyl)butanoic acid was used to attach on the peptide. After the last coupling step, excessive reagents were removed by a single DMF wash for 5 minutes (5 mL per gram of resin), followed by five steps of washing using DCM for 2 min (5 mL per gram of resin). The peptide derivative was cleaved using 95% of trifluoroacetic acid with 2.5% of TMS and 2.5% of H_2O for 20 minutes. 20 mL per gram of resin of ice-cold diethylether was then added to cleavage reagent. The resulting precipitate was filtrated and washed by ice-cold diethylether. The resulting solid was further purified by HPLC and dried by lyophilizer.

Synthesis of PhSe$(CH_2)_3$COFFGK(Van)(Compound 1): The compound of PhSe$(CH_2)_3$COFFGK (100 mg) was dissolved in 8 mL of DMSO, 2 equivalent of vancomycin (401 mg) and HBTU (104 mg) was added. The pH of the mixture solution was then adjusted to about 9 by DIEA. After 8 hours reaction, the crude product was purified by HPLC and dried by lyophilizer with total yield 34%.

Oxidation of PhSe$(CH_2)_3$COFFGK(Van)(Compound 2): To a solution of selenide peptide, 200 mg (0.21 mmol) of PhSe$(CH_2)_3$COFFGK(Van) in the mixture of MeCN (5 mL) and H_2O (5 mL), 48 μL of 30% H_2O_2 (2 eq.) was added. The mixture was stirred at room temperature for 12 h. LC-MS spectra indicated that the reaction had converted completely. MeCN was then removed by vacuum and selenoxide peptide was obtained equivalently by freeze-drying.

Compound PhSe$(CH_2)_3$COFFGK: ^{1}H NMR (300 MHz, DMSO) δ 8.22 (s, 2H), 8.15 – 8.05 (m, 3H), 7.70 (s, 3H), 7.41 – 7.37 (m, 2H), 7.26 (d, J = 1.5 Hz, 1H), 7.25 – 7.20 (m, 6H), 7.17 (d, J = 4.0 Hz, 5H), 4.48 (d, J = 8.6 Hz, 3H), 4.20 (d, J = 4.2 Hz, 2H), 3.74 (s, 2H), 3.05 (dd, J = 13.9, 4.2 Hz, 2H), 2.94 (dd, J = 13.7, 4.2 Hz, 2H), 2.72 (s, 1H), 2.69 (d, J = 3.8 Hz, 1H), 2.10 (t, J = 7.2 Hz, 2H), 1.68 (dd, J = 16.7, 7.4 Hz, 4H), 1.52 (d, J = 7.5 Hz, 3H), 1.35 (d, J = 7.0 Hz, 3H).

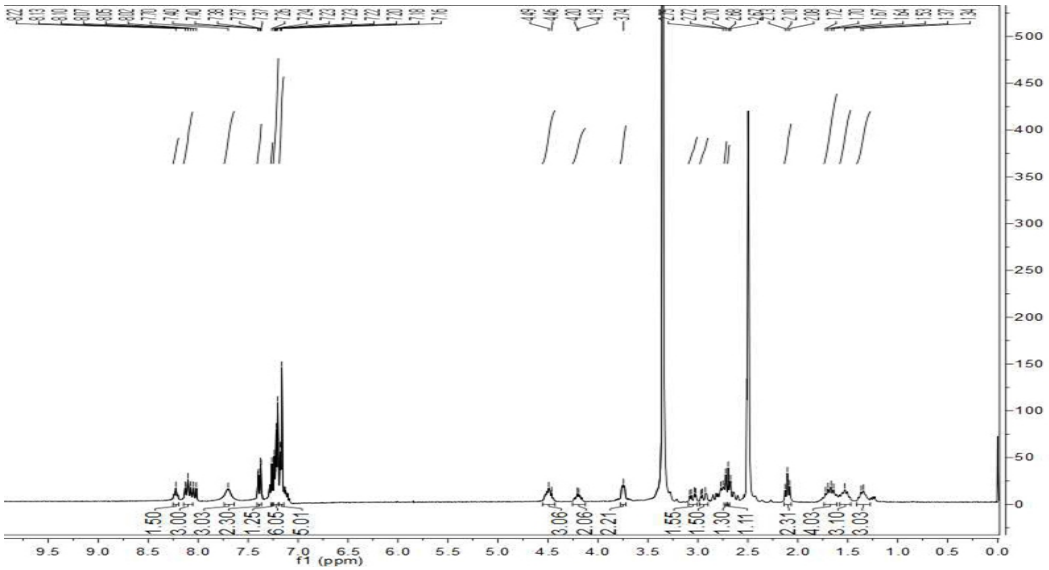
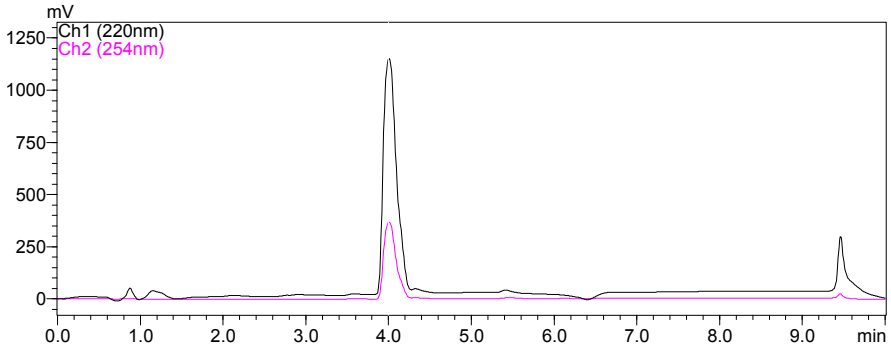


Figure S-2. ^{1}H NMR of ***PhSe$(CH_2)_3$COFFGK***



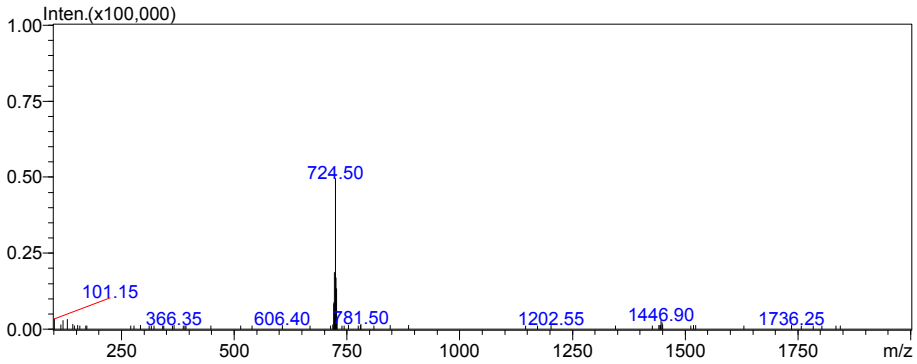


Figure S-3. LC-MS of ***PhSe(CH$_2$)$_3$COFFGK***

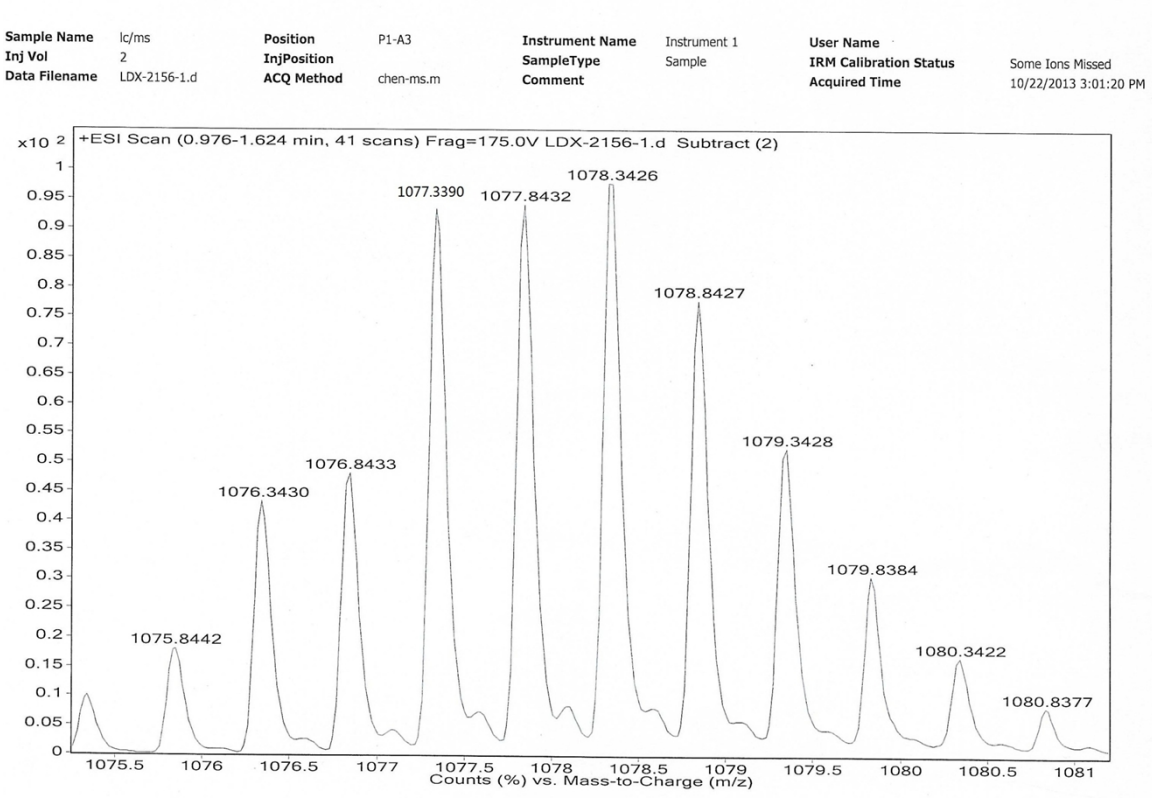


Figure S-4. HR-MS of Compound ***1***

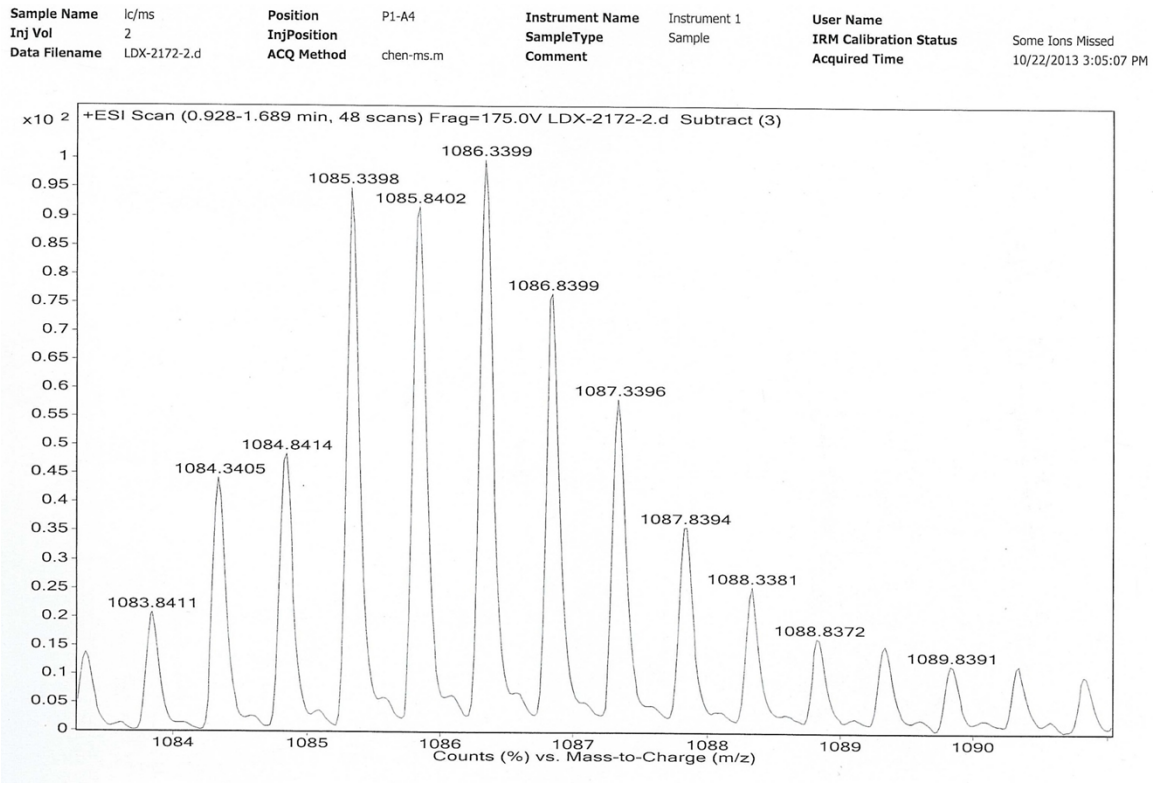


Figure S-5. HR-MS of Compound ***2***

LC-MS analysis for the conversion from compound *1* to compound *2*: After treating with compound ***1*** with 30% H_2O_2 (2 equiv.), we prepared samples for the LC-MS analysis. The samples were used for the analysis at different times and the total samples for each measurement was three. The areas of peaks in LC-MS spectra were used to determine the conversion percentage from compound ***1*** to compound ***2***.

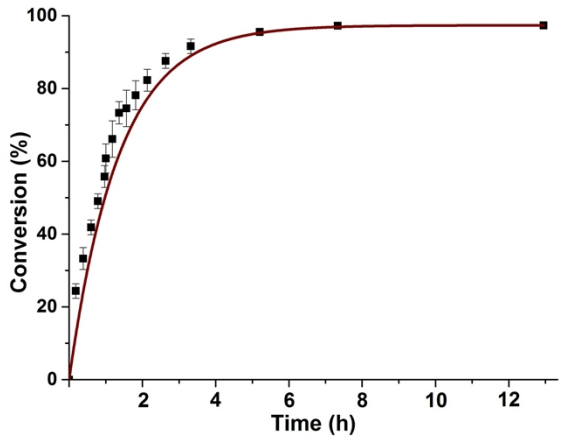


Figure S-6. Conversion of compound ***1*** to compound ***2***

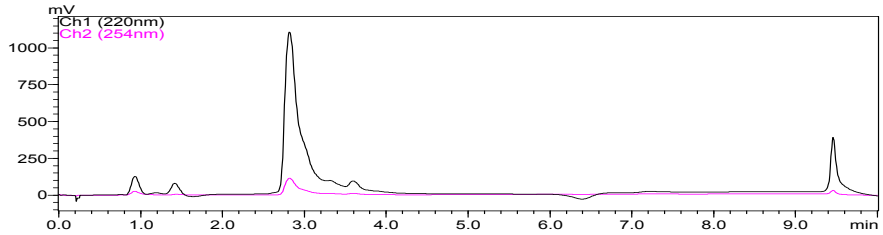


Figure S-7. The HPLC spectra of the solution of compound **1** after being treated with H_2O_2 for 24 hours

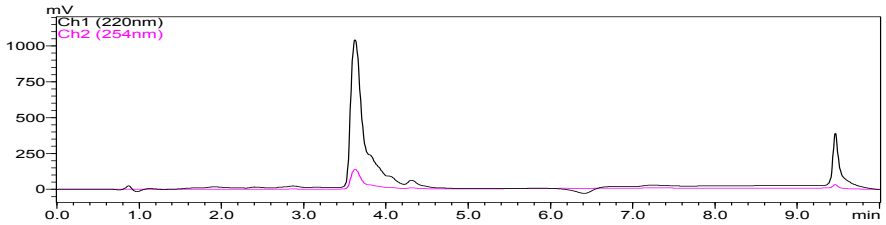


Figure S-8. The HPLC spectra of the solution of compound **2** after being treated with VC for 4 hours

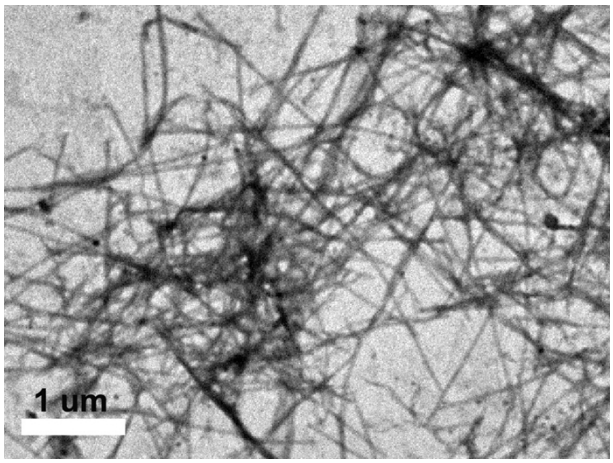


Figure S-9. TEM image of the solution of oxidated compound ***1*** in PBS (0.1 wt%) treating with 4 equivalent of VC for 12h

MIC test procedure: A standard broth dilution method was used to determine the MICs of bacteria. Compound ***1***, ***2*** and vancomycin hydrochloride were dissolved in DMSO to obtain 100 mg/mL stock solution, respectively. A total of 100 μL of LB solution was added to a series of holes on a sterile 96 well plates, with an additional 98 μL of LB solution added to the first one. 2 μL of a 100 mg/mL compound stock solution was added to the first hole, and a series of 2-fold dilutions were prepared by transferring 100 μL to successive tubes. 5 μL of bacterial solution at an OD_{600} value of 0.5 was added to each hole containing different concentration of compounds. The compound-treated cultures were incubated at 37°C for 24 h, and the OD_{600} was measured. Each measurement was performed in triplicate.

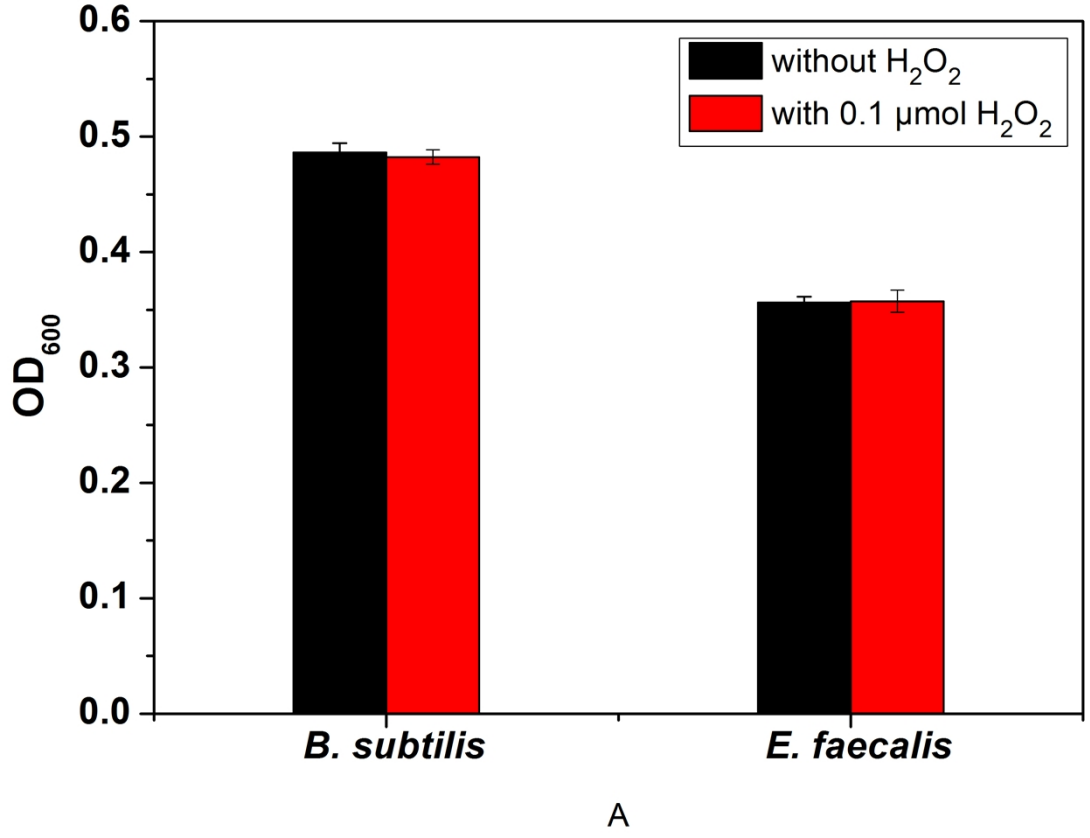


Fig. S-10. MIC test results of B. Subtillis and E. Faecalis treated by 0.1μmol of H_2O_2.