In vitro antimicrobial assay of nano-iron sulfide (nFeS)

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Method Article

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Abstract

This protocol describes a detailed process of antibacterial \(Streptococcus mutans UA159) activity by nanometer-sized iron sulfide \(nFeS). Streptococcus mutans has long been considered to be a critical factor in caries-causing plaque development. The pathogens are enriched in 3D matrix of extracellular polymeric substances and lead to dental caries by producing highly acidic niches. We find that nano-iron sulfide improves the killing efficacy of Streptococcus mutans UA159 by more than 500 times compaired with garlic-derived organosulfur compounds. Here the Fe3O4 nanoparticles and nFeS\(Cys-nFeS, GSH-nFeS, NaHS-nFeS, Cyss-nFeS, and DADS-nFeS) converted from different organosulfur sources were used to evaluate the antimicrobial \(Streptococcus mutans UA159) activity within 30 minutes.

Reagents

ethyl alcohol, tryptone, yeast extract, glucose, 0.1 M sodium acetate \(pH 4.5)

Procedure

Bacteria culture and inoculum preparation 1. Cryopreserved Streptococcus mutans UA159 were thawed from a frozen stock, and spread on tryptone-yeast extract ager broth with 1% glucose. Then the plate was incubated at 37°C and 5% CO_2 for 48 h. 2. Transfer several colonies with inoculation loop and place into 15 mL of sterile tube, resuspend, and incubated the pre-culture at 37°C and 5% CO_2 for 12-16 h. 3. Transfer 200 µL of overnight cultured bacteria solution to 3.8 mL fresh tryptone-yeast extract broth with 1% glucose and cultured for 4 h when the optical density \(OD) at 600 nm was about 1.0. CAUTION: Streptococcus mutans UA159 on ager broth stored at 4°C was easy to lose activity. Choose the bacteria on the ager plate stored within two weeks, and pick several colonies for activating bacteria overnight. 4. Harvest the bacteria by centrifugation at 5000 g for 5 min. 5. Wash the bacteria two times by resupending the pellet into 0.1M NaAc \(pH 4.5) and centrifugr two times at 5000 g for 5 min. 6. Resuspend the pellet into 0.1M NaOAc \(pH 4.5) 7. Adjust the bacteria population to 10^8 CFU/mL. **Antimicrobial preparation** 1. Wash the Cys-nFeS, DADS-nFeS nanoparticles with ethyl alcohol 3 times, respectively. 2. Resuspend the nanoparticles into ddH_2O and magnetic separation for 3 times. 3. Autoclave the nanoparticles by 121°C, 20 min. 4. Dilute the autoclaved nanoparticles to 5 mg/mL. **In vitro antimicrobial assay** 1. Add 100 µL of bacteria into 900 µL of 0.1 M sodium acetate \(pH 4.5) and resuspend the mixture. Dilute and plate \(at least 3 dilutions per sample) accordingly. 2. Add 100 µL of bacteria and 100 µL of 5 mg/mL Cys-nFeS nanoparticles into 800 µL of 0.1 M sodium acetate \(pH 4.5), and resuspend the mixture. Dilute and plate \(at least 3 dilutions per sample) accordingly. 3. Add 100 µL of bacteria and 100 µL of 5 mg/mL DADS-nFeS nanoparticles into 800 µL of 0.1 M sodium acetate \(pH 4.5), and resuspend the mixture. Dilute and plate \(at least 3 dilutions per sample) accordingly 4. Count colonies 24-48 h later and plot them.

Anticipated Results

1. Colony counts of less than 20 colonies or more than 350 colonies per plate were not considered. 2. Streptococcus mutans UA159 can be killed significantly by iron sulfide nanoparticles in 0.5 mg/mL within 30 min.

Figures

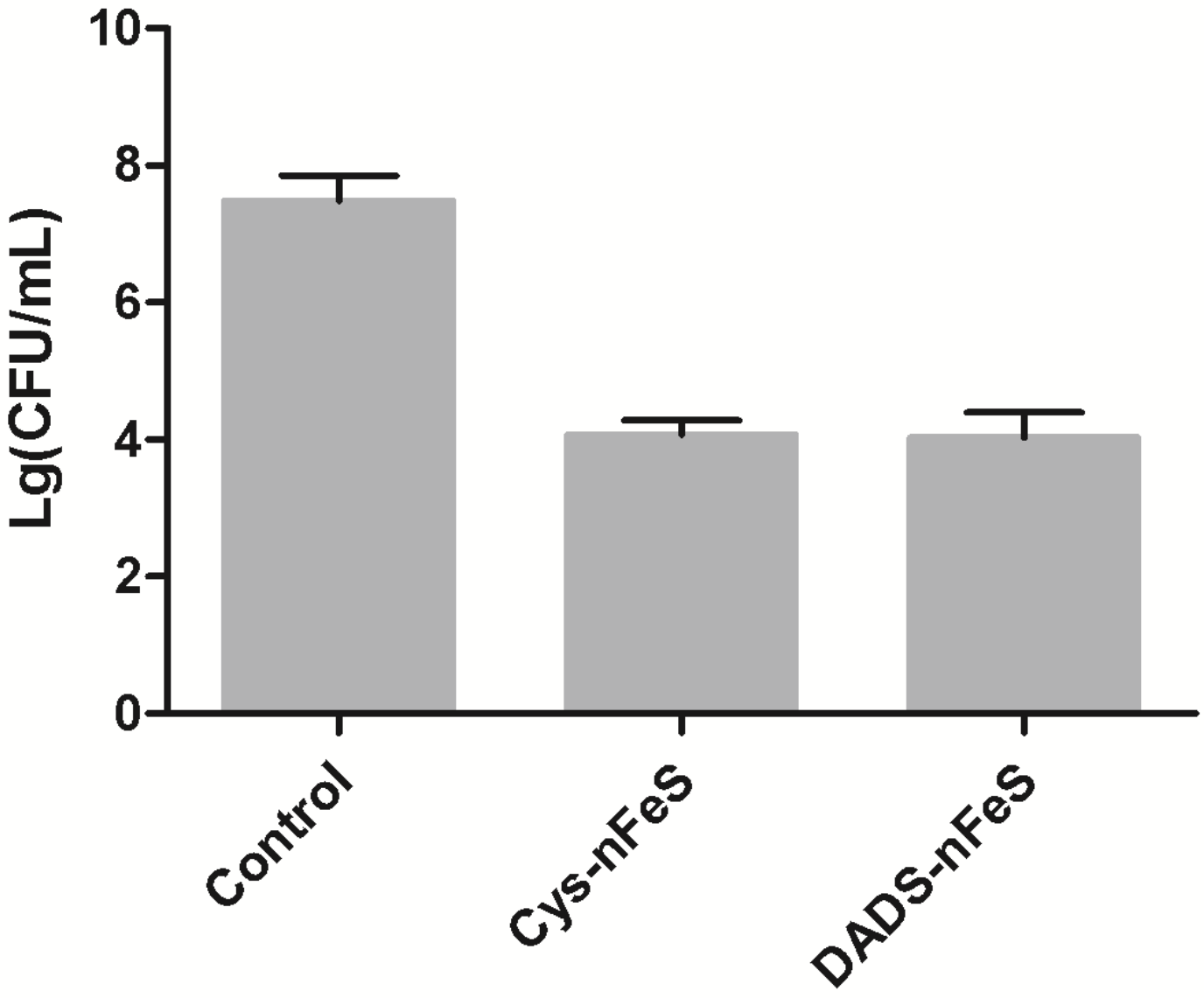

Figure 1

Figure1 anti-bacterial (S.mutans UA159) activity of Cys-nFeS and DADS-nFeS S.mutans inhibition of Cys-nFeS and DADS-nFeS. 0.1 M sodium acetate (pH 4.5) was used as control. Both the concentration of Cys-nFeS and DADS-nFeS are 0.5mg/mL.