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Antimicrobial effects of quaternary phosphonium salt intercalated clay minerals on *Escherichia coli* and *Staphylococci aureus*

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ABSTRACT

The aim of this research was to determine the antimicrobial properties and influence factors of four clay minerals intercalated by quaternary phosphonium salt (tetradecyl tributyl phosphonium bromide, TDTB) on antimicrobial effects. *Escherichia coli* (*E. coli*) and *Staphylococci aureus* (*S. aureus*) were chosen for Minimal Inhibitory Concentration (MIC) tests to evaluate the antimicrobial activities of organ-clay minerals. The properties of organ-clay minerals were analyzed by FTIR, XRD, TEM, Z-Average and Zeta potential, and the releasing amount of TDTB into the broth was measured by ICP. The results showed that montmorillonites-TDTB inhibited the growth of *E. coli* and *S. aureus*, and the MICs were 200 ± 20 and 80 ± 15 mg/L, respectively. The amount of TDTB released into the broth was $3.42 \pm 0.71\%$. According to the properties of organ-clay mineral, it was indicated that the antimicrobial activities of organ-clay mineral were the synergic effect of the releasing amount of TDTB, Zeta potential, particle size and distribution. The organ-clay mineral with the larger releasing amount of TDTB, the higher Zeta potential, the same particle size with bacterial size and the narrower size distribution exhibited better antimicrobial activity. The present study demonstrated the properties of organ-clay minerals effected their antimicrobial activities, provided theoretically guidance to promote the antimicrobial activity of clay minerals.

1. Introduction

In order to resolve the problems of microbial contamination, researchers have intensively investigated in antimicrobial materials containing various original and synthetic substances [1]. For example, inorganic antimicrobial materials carrying Ag^+ act as the most important antimicrobial materials [2–4], other metal ions such as Cu^{2+} and Zn^{2+} can also be employed as antimicrobial materials [5–7]. Nevertheless, the applications of inorganic antimicrobial materials are limited because an accumulation of heavy metals will result in serious environmental problems and may be harmful to humans in the case of high metal concentration [8]. On the other hand, inorganic antimicrobial materials can form insoluble clay minerals and then easily lose their antimicrobial activity [5]. Hence, it is important to develop new antimicrobial materials with low cost, high antimicrobial activity and light permanency.

Comparing to inorganic antimicrobial materials, clay minerals have attracted much more attention due to their non-toxic, environmentally friendly characteristics and easily prepared by, e.g., their intercalation with selected organic [9,10] or inorganic [3,9] substances with antimicrobial properties, widely antimicrobial spectra, high security and the synergistic effect with other antimicrobial materials [11]. And the antimicrobial effects by which several clay minerals act are notably explained as follows: (1) interaction phenomena between organic molecules [12] (e.g., humic acid), inorganic ions and microorganisms; (2) the formation of C–O–Na–Si complexes on the surfaces of bacterial cell walls [13,14]; (3) drug loadings of modified clays [3,8]; and (4) particle size of clay minerals [15,16].

Our current paper intended to explore the influence factors of organ-clay minerals on antimicrobial activities. Herein, four clay minerals were intercalated by quaternary phosphonium salt, and then were used to evaluate their antimicrobial activities against *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*). Then, the relationships between the antimicrobial activity and the properties of organ-clay minerals, such as releasing amount of quaternary phosphonium salt, particle size and Zeta potential were studied. The present study will provide the theoretical guidance to promote the antimicrobial activity of antimicrobial agents based clay minerals.

2. Materials and methods

2.1. Materials

Montmorillonites (MMT) with 110 meq/100 g cation-exchange capacity (CEC) was obtained from Fenghong New Material Co., Ltd. (Zhejiang, China). Vermiculite (VER) and palygorskite (PAL)

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were supplied by Anji Clay Co., Ltd. (Zhejiang, China). Kaolin (KAO) was purchased from Datong Coal Industrial Jinyu Kaolin Chemical Co., Ltd. (Shanxi, China). VER, PAL and KAO were sodiumized in laboratory, and their CEC were 98.5 meq/100 g, 65 meq/100 g and 97.3 meq/100 g, respectively. All of the clay minerals were stored in a sealed bottle for further use. The quaternary phosphonium salt, tetradecyl tributyl phosphonium bromide (TDTB), was purchased from Qingte Chemical Industry Co., Ltd. (Shanghai, China). Mueller–Hinton broth and nutrient agar culture medium were supplied by Huankai Microorganism Co., Ltd. (Guangzhou, China). *E. coli* ATCC 25922 and *Staphylococci aureus* ATCC 6538 were supplied by Guangdong Institute of Microbiology (Guangzhou, China).

2.2. Sample preparation

The preparation of organ-clay minerals was carried out by the following process: 10 g of clay mineral was dispersed in 490 g of deionized water, to which TDTB was slowly added. The quantity of TDTB was at an amount of 2.0 times the CEC of clay mineral. Then reagents were stirred vigorously at 65 °C for 6 h. Then, the organ-clay mineral was washed with deionized water until the washing liquor with 1% $AgNO_3$ solution was negative. After dried at 65 °C under vacuum, the organ-clay mineral was collected with 300 mesh sieve (48 μm). The resulting organ-clay minerals were named as KAO-TDTB, MMT-TDTB, VER-TDTB and PAL-TDTB, respectively.

2.3. Antimicrobial activity assay

The antimicrobial activity of clay minerals and organ-clay minerals were evaluated by determining the minimum inhibitory concentrations (MICs) by two-fold serial dilutions of clay minerals and organ-clay minerals in the Mueller–Hinton broth, using the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS, 2000). Each 1 mL of culture medium containing various concentrations of test sample was inoculated with 0.1 mL of 10^7 cfu/mL bacterial suspension (determined by "standard plate count"), cultured for 24 h at 37 °C under shaking, and then the growth of bacteria was observed. MIC values were determined as the lowest concentration of the tested sample where the absence of growth was recorded [17]. Three repeats were carried out for each test.

2.4. Instruments and techniques

Fourier transformed infrared spectrometer (FTIR) spectra between 400 and 4000 cm^{-1} were collected on a Nicolet 6700 spectrometer. X-ray powder diffraction patterns were recorded on a Rigaku D/Max 1200 X-ray Diffractometer, using Cu $K\alpha$ radiation (λ = 0.1541 nm), operating at 40 kV and 40 mA with a scan speed of 1°/min and 2θ range of 2.0–40.0°. Particle size (Z-Average) and Zeta potential were determined using a PALS Zeta Potential Analyzer. Transmission electron microscopy (TEM) was performed on Philips TECNAI-10 transmission electron microscope with an accelerating voltage of 200 kV.

2.5. Release of TDTB from organ-clay minerals

The releasing amount of inserted TDTB into MH broth was examined. Organ-clay minerals dilution broth was prepared in the same way as the antimicrobial test. At each time-point (0.5, 2, 4, 8, 12 and 24 h), 12 tubes containing broth of four different organ-clay minerals (three tubes for each organ-clay mineral) were taken out of the culture box and centrifuged at 4000 rpm for 10 min, and their supernatants were collected separately. The concentrations of phosphorus of TDTB in the supernatants were measured using Inductive Coupled Plasma Atomic Emission Spectrometer (ICP-AES, Optima 2000 DV, PerkinElmer Inc.). The samples were pH determined before being acidified to pH = 1.00–2.00 with 1 mol L^{-1} HNO_3. After 1 h of acidification (shaking intermittently), solutions were centrifuged at 10,000 rpm for 10 min [18]. After centrifugation, the supernatants were extracted for determining the concentrations of dissolved phosphorus (test limits: 110 nmol L^{-1}; test power: 1.2 kW, plasma gas: 15 L min^{-1}; auxiliary gas: 0.2 L min^{-1}; nebulizer gas: 0.8 L min^{-1}; sample uptake: 1.5 mL min^{-1}; detection wavelength: 178.2 nm).

Table 1
MICs for the effects of TDTB, original and organ-clay minerals on *E. coli* and *S. aureus*.

Samples	[a]MIC/mg L^{-1}	
	E. coli	*S. aureus*
TDTP	16 ± 3	6 ± 2
KAO	>10,000	>10,000
KAO-TDTB	400 ± 40	100 ± 15
MMT	>10,000	>10,000
MMT-TDTB	200 ± 20	80 ± 15
VER	>10,000	>10,000
VER-TDTB	750 ± 30	120 ± 25
PAL	>10,000	>10,000
PAL-TDTB	800 ± 50	150 ± 20

[a] ±SD, n = 4.

3. Results and discussion

3.1. Antimicrobial activity of original and organ-clay minerals

The MIC of TDTB, original and organ-clay minerals on *E. coli* and *S. aureus* were shown in Table 1. MMT-TDTB displayed the highest antimicrobial activity in four organ-clay minerals, with 200 ± 20 mg L^{-1} and 80 ± 15 mg L^{-1} of MIC on *E. coli* and *S. aureus*, respectively. However, both of the bacteria still grew in the broth containing 10,000 mg L^{-1} of four original clays. This reflects that original clays have no antimicrobial ability, while organ-clay minerals could significantly inhibit growth of the tested bacteria.

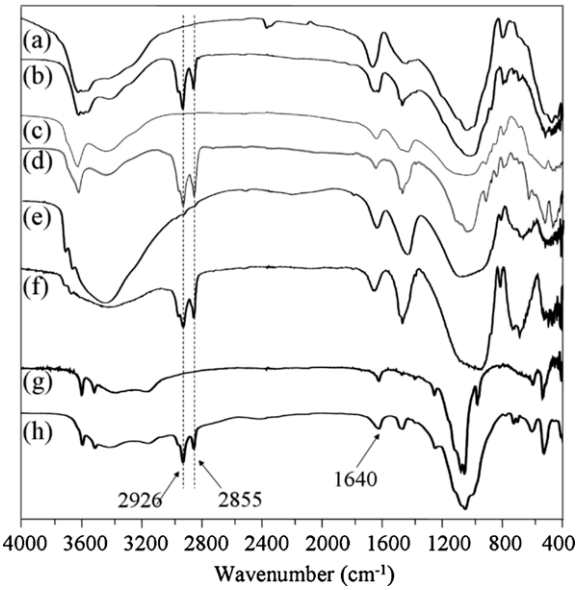

Fig. 1. FTIR spectra of original and organ-clay minerals:(a) KAO-TDTB; (b) KAO; (c) MMT-TDTB; (d) MMT; (e) VER-TDTB; (f) VER; (g) PAL-TDTB; and (h) PAL.