

Supplementary Information for

Phosphonium-based ionic liquids as antifungal agents for conservation of sandstone heritage

Qiang Li^{1*}, Yulan Hu¹, Bingjian Zhang^{1,2*}

¹School of Art and Archaeology, Zhejiang University, Hangzhou, Zhejiang 310058,
China

²Department of Chemistry, Zhejiang University, Hangzhou, Zhejiang 310027, China

*Corresponding author: E-mail: zhangbiji@zju.edu.cn; 11437071@zju.edu.cn

1.1 XTT-reduction assay

XTT-reduction assay was used to determine the in vitro activity of ILs against fungal biofilms. Briefly, four fungal isolates were grown for 7 d in PDA medium at 30°C. The fungal aggregates were filtered through a filter paper and the spores were harvested and washed three times with phosphate-buffered saline (PBS). The spore suspensions were then diluted in RPMI 1640 medium to a concentration of 1×10^5 CFU/mL, and 150 μ l was transferred into each well of 96-well tissue culture plates. The plates were incubated at 30°C for 2 - 3 d until the biofilm was visible. After biofilm formation, the medium was carefully removed and the biofilm was washed with PBS three times to remove residual medium and nonadherent fungal cells. Subsequently, 200 μ l of freshly prepared RPMI 1640 medium containing a final concentration of 0.05, 0.1, 0.5, 1, 5, and 10 mg/mL of ILs was added to each well, whereas medium containing 1% DMSO was used as a control group. The fungal cells were further incubated at 30°C statically for 24 h, after which the biofilm was carefully washed with PBS three times. Finally, 100 μ l of the XTT (0.5 mg/mL)/menadione (1 μ M) solution was added to each well and the plates were incubated in the dark for 2.5 h at 37°C. A volume of 80 μ l of the colored supernatant from each well was used to determine the absorbance at 490 nm.

Table S1 Elemental analysis of $K_8[\alpha\text{-SiW}_{11}\text{O}_{39}]\cdot 13\text{H}_2\text{O}$ and three ILs. The values are given as mass percentages (%).

	calculated			experimental		
	C	H		C	H	
$[\text{P}_{44412}][\text{POM}]$	40.82	7.37		41.51	7.78	
$[\text{P}_{44414}][\text{POM}]$	42.53	7.63		42.82	7.78	
$[\text{P}_{66614}][\text{POM}]$	46.97	8.32		45.86	7.94	
	K	Si	W	K	Si	W
$K_8[\alpha\text{-SiW}_{11}\text{O}_{39}]\cdot 13\text{H}_2\text{O}$	9.71	0.87	62.78	9.12	0.86	62.23

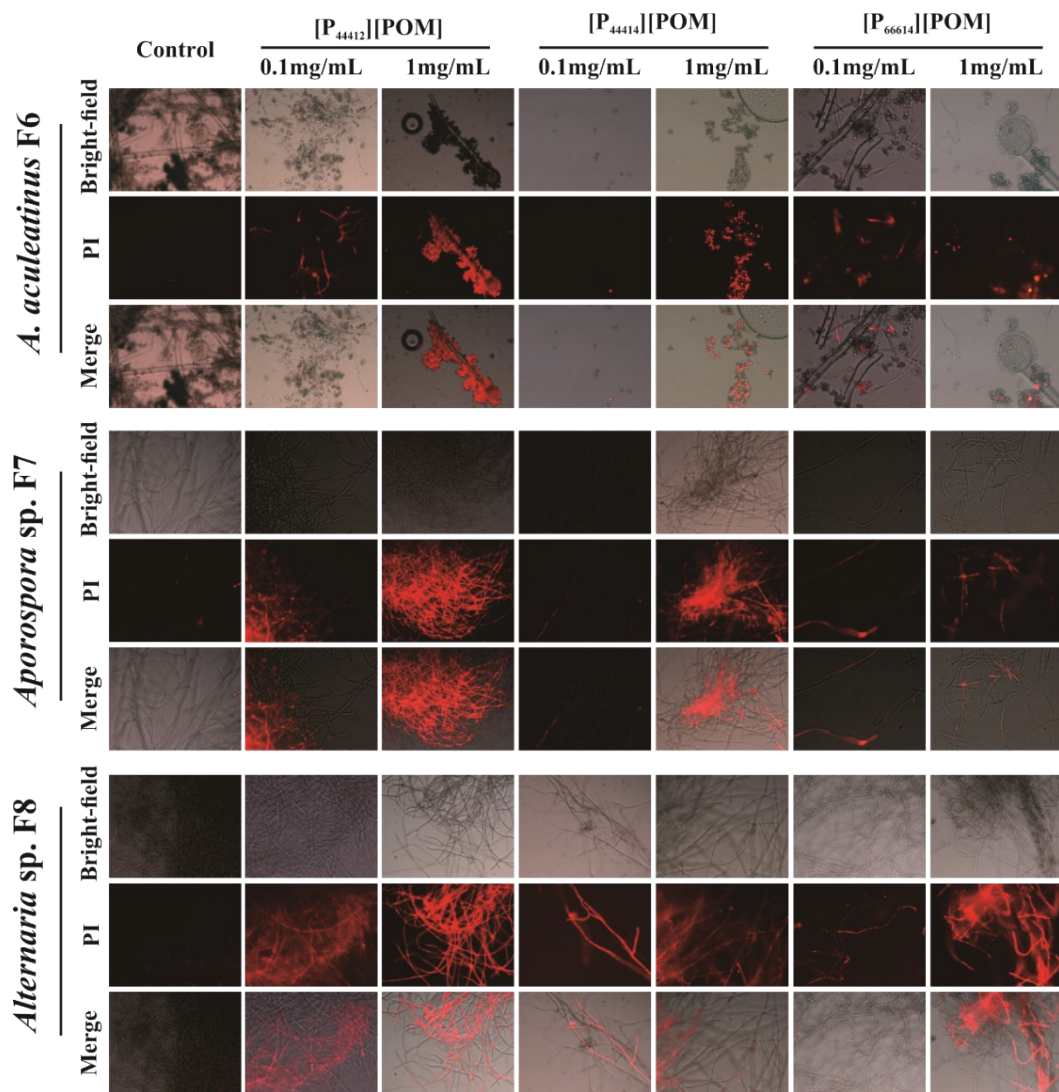


Fig. S1 Effect of 0.1 and 1 mg/mL of ILs on the membrane integrity of *A. aculeatinus* F6, *Aporospora* sp. F7, and *Alternaria* sp. F8.

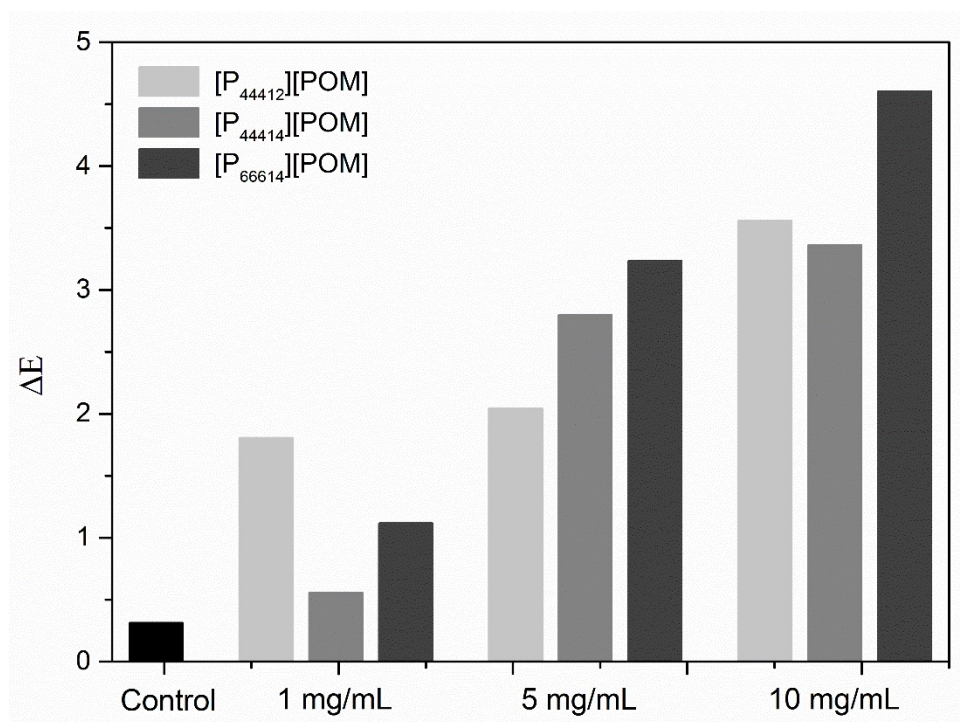


Fig. S2 Color difference values (ΔE) for the sandstone samples, before and after the treatment with [P₄₄₄₁₂][POM], [P₄₄₄₁₄][POM], and [P₆₆₆₁₄][POM].