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RESEARCH NOTE

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Synergistic Antimicrobial Action of Thymol and Sodium Bisulfate against *Burkholderia cepacia* and *Xanthomonas maltophilia* Isolated from the Space Shuttle Water System

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Abstract A combination of thymol and sodium bisulfate was found to be an effective biocidal agent against strains of *Burkholderia cepacia* and of *Xanthomonas maltophilia* that were found in the space shuttle water system. Potassium iodide (KI), the biocidal agent used in the past, had a minimum inhibitory concentration (MIC) of 50,000 ppm against the two *B. cepacia* (541 STS-81 and 1119 STS-91) strains, whereas that of thymol and sodium bisulfate was 2,400 and 950 ppm, which was 21 and 53 times lower than that of KI for *B. cepacia*, respectively. The MIC value for the combination of thymol and sodium bisulfate was 4 times lower than that for thymol or sodium bisulfate alone against *B. cepacia* (541 STS-81, 1119 STS-91) or *Pseudomonas cepacia* (ATCC 31941). The fractional inhibitory concentration (FIC) of the combination of thymol and sodium bisulfate for all organisms tested was less than 0.5, indicating a strong synergistic effect.

Keywords: antimicrobial aeTion, *Burkholderia cepacia, Xanthomonas maltophilia*

Introduction

Burkholderia cepacia was first reported by Burkholder as a pathogenic agent causing spoilage in onion bulbs (1). This organism was isolated from the rhizosphere of maize (2), water and sediments of streams (3), and soil (4). *B. cepacia* has emerged as an opportunistic pathogen causing pulmonary infections in patients with cystic fibrosis (5, 6). *B. cepacia* was isolated from the tubing system in the space shuttle Discovery. The quality of spacecraft water is an important factor for the safety of the crew working for long periods in space. Both mechanical and chemical methods have been applied to reduce biofilm contamination of water lines but have been ineffective in eliminating rapid reinfection (7). Day *et al.* (8) reported that a complex of hydrogen peroxide, thymol, and sodium bisulfate, which are all classified as 'generally regarded as safe' (GRAS), was successful in decontaminating thick *Pseudomonas aeruginosa* biofilms. Iodine products have been used to control chronic contamination of the portable water system on the space shuttle (9, 10). However iodine is only effective against planktonic cells. The biocidal effects of a combination of thymol and sodium bisulfate were tested against *B. cepacia* and *Xanthomonas maltophilia* that had been isolated from water lines of the space shuttles and their effectiveness was compared with the biocidal effectiveness of potassium iodide (KI) against *B. cepacia* and *X. maltophilia*.

Materials and Methods

Bacteria strains Four bacterial strains were used in this study: *B. cepacia* 541 STS-81, *B. cepacia* 1119 STS-90, *X. maltophilia* STS-84, which was isolated originally from a spacecraft water system and was a gift from Dr. C. M. Ott at the Johnson Space Center, (Houston, TX, USA), and *Pseudomonas cepacia* (ATCC 31941) which was obtained from the American Type Culture Collection (Rockville, MD, USA). Nutrient broth (Difco, Sparks, MD, USA) was used in all experiments.

Biocide solutions KI, thymol and sodium bisulfate were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Dilutions of the stock solution (2.4% sodium bisulfate and a saturated solution of thymol) were prepared in all experiments prior to use.

Measurement of antimicrobial activity The minimum inhibitory concentration (MIC) values in broth culture for the individual components and combination as a biocide were determined. Serial dilutions of test compounds, in 5 mL of nutrient broth, were inoculated with aliquots of individual cultures sufficient to produce initial counts of 10^6 cfu/mL. The dilution series were incubated for 24 hr at 25°C. MIC was defined as the lowest concentration of biocide by measuring absorbance at 600 nm where there was an absence of growth after 24 hr (11-13). Fractional inhibitory concentrations (FICs) were calculated based on the method of Berenbaum (14).

Results and Discussion

The MIC values for KI, thymol, sodium bisulfate ($NaHSO_4$), and the combination of thymol and sodium bisulfate against the test organisms are shown in Table 1. KI had an MIC of 50,000 ppm for the *B. cepacia* (541 STS-81 and 1119 STS-91) strains. *P. cepacia* and *X. maltophilia* STS-84 all were resistant to KI with MIC values ranging from 60,000 to 70,000 ppm. The MIC values for thymol were 20-50 times lower than for KI for

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Table 1. Minimum inhibitory concentrations (MIC) of potassium iodide (KI), thymol, sodium bisulfate ($NaHSO_4$) and the combination of thymol and sodium bisulfate against four bacteria strains found in the space shuttle water system

Organisms	MIC (ppm)				
	Single component			Combination[3]	
	KI	Thy[1]	SB[2]	Thy[1]	SB[2]
Pseudomonas cepacia ATCC 31941	60,000	1200	480	480	120
Burkholderia cepacia 541 STS-81	50,000	2400	950	950	240
Burkholderia cepacia 1119 STS-91	50,000	2400	950	950	240
Xanthomonas maltophilia STS-84	70,000	2400	480	480	120

[1]Thymol
[2]Sodium bisulfate ($NaHSO_4$)
[3]MIC (ppm) of each component in a mixture of biocide solutions, thymol and sodium bisulfate. Data are means which have a standard deviation (±10) obtained from triplicate experiments (n=9).

these strains. Sodium bisulfate was the most effective in killing the test organisms of the components tested. The MIC values for sodium bisulfate were 480 ppm against both *P. cepacia* (ATCC 31941) and *X. maltophilia* STS-84 and 950 ppm against *B. cepacia* (541 STS-81, 1119 STS-91). MIC for sodium bisulfate was 53 times lower than that for KI against both *B. cepacia* strains (541 STS-81, 1119 STS-91) and 146 times lower against both *P. cepacia* (ATCC 31941) and *X. maltophilia* STS-84. The greatest biocidal effects were observed with a combination of thymol and sodium bisulfate. MIC for the combination of thymol and sodium bisulfate was 4 times lower than that of thymol or sodium bisulfate alone against all strains. FIC of the two-component mixture was less than 0.5 for all the organisms tested, indicating a strong synergistic effect. The synergistic action of the combination of thymol and sodium bisulfate may have resulted from the combination of cell membrane perturbation and permeability loss by thymol (15), the limitation of bacterial growth and the inactivation of essential enzymes such as DNA polymerase I for the repair of denatured proteins by sodium bisulfate (16).

Although significant progress has been made in developing compositions that are effective for decontaminating water lines, each of the new treatments has some disadvantages. Treatments using detergents require sufficient rinsing for safe use of water lines (8). Peracetic acid produces volatile compounds with disagreeable odors (8, 17). Sodium hypochlorite may result in corrosion of in-line metal valves (18) and it is not particularly effective against biofilms. The quinolone class of antimicrobial agents has an extensive antibacterial spectrum, but the use of quinolones such as temafloxacin was banned in 1992 because they were associated with hepatic toxicity (19). The study results presented here demonstrated that a specific combination of thymol and sodium bisulfate exhibited an effective synergistic action against both *B. cepacia* and *X. maltophilia*. The use of this combination may provide a safe and effective substitute for the current antimicrobial agents used for disinfecting water systems.

Table 2. Fractional inhibitory concentrations (FIC) of the combination of thymol and sodium bisulfate against four bacteria strains found in the space shuttle water system

Organisms	FICs
Pseudomonas cepacia ATCC 31941	0.5
Burkholderia cepacia 541 STS-81	0.5
Burkholderia cepacia 1119 STS-91	0.3
Xanthomonas maltophilia STS-84	0.4

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