

ANTIMICROBIAL TESTING

E-Coli K-12 - E-Coli 0157:H7

**Salmonella Enterica Servoar Typhimurium LT2
Enterococcus Faecalis**

**Staphylococcus Aureus (Staph Infection MRSA)
Streptococcus Pyrogenes**

**Anti Bacteria effect of E-Coli 0157:H7 on
Spinach leaves**

By: Texas Tech University

FINAL RESEARCH REPORT
Antibacterial Susceptibility Test Results for the Alka-Hydroxy® Product
July 17, 2006

The **purpose** of this series of experiments was to test the susceptibility of both Gram-positive and Gram-negative bacteria to the modified liquid silicon product Alka-Hydroxy®.

Six **organisms** were tested. The Gram-negative bacteria were *Escherichia coli* K-12, *Escherichia coli* O157:H7 (ATCC 35150), and *Salmonella enterica* serovar Typhimurium LT2. The Gram-positive bacteria were *Enterococcus faecalis* (ATCC 19433), ***Staphylococcus aureus* (MRSA)** (ATCC 12600), and *Streptococcus pyogenes* (ATCC 19615).

The **method** used was the broth microdilution method for susceptibility testing of antibacterial agents as recommended by the Clinical and Laboratory Standards Institute (CLSI). The detailed procedure is as follows.

1. A culture of the test organism was grown at 35°C with aeration in sterile Cation-adjusted Mueller-Hinton broth (Becton-Dickinson, Sparks, MD) until slightly turbid, and the turbidity was then adjusted to a 0.5 McFarland standard [equivalent to approximately 10⁸ colony-forming units (cfu) per ml]. To improve cell yield in the *Enterococcus faecalis* and *Streptococcus pyogenes* cultures, the Cation-adjusted Mueller-Hinton broth was supplemented with 2.5 mg per mL of yeast extract (Becton-Dickinson).
2. Appropriate dilutions of the Alka-Hydroxy® product were prepared in sterile Cation-adjusted Mueller-Hinton broth such that the final concentrations of the product were 0.5%, 1.0%, 2.0%, 3.0%, and 5.0%. A 0% (no-product) control was also included.
3. Appropriate volumes of the standardized cell suspension were added to the dilutions of the product to achieve a final cell density of 5 x 10⁵ cfu per mL. The dilutions were then aliquoted into the wells of a 96-well microtiter plate (with a volume of 0.2 mL per well).

4. The first two wells in each column of the microtiter plate were reserved for a no-cell control, and the remaining six wells were used for the cell-inoculated dilutions of the product. Columns 1–6 represented the 0%, 0.5%, 1.0%, 2.0%, 3.0%, and 5.0% concentrations, respectively.
5. The microtiter plates were incubated at 35°C for 16–20 hours. The culture turbidity was measured by visible-light spectroscopy (i.e., the absorbance of the culture at a wavelength of 590 nm) using a microtiter plate reader, and the results were recorded automatically.
6. The baseline absorbance of the wells containing the uninoculated dilutions (no-cell controls) was subtracted from the absorbances of the wells containing the cell-inoculated dilutions of the product. For each set of six replicate wells, the average (mean) absorbance, % inhibition, and standard deviation were calculated from the collected data.

The **results** of the experiments are summarized in the appended data tables. The minimum inhibitory concentration (MIC) of the product was the 1.0% concentration for all three of the Gram-negative bacteria (*E. coli* K-12, *E. coli* O157:H7, and *S. enterica*). However, the three Gram-positive bacteria (*E. faecalis*, *S. aureus*, and *S. pyogenes*) showed greater resistance. The MIC of the product was the 2.0% for *S. aureus* and 3.0% for *E. faecalis* and *S. pyogenes*, although partial inhibition was seen at lower concentrations for all three of these organisms.

The **conclusions** drawn from the experimental results are that the product completely inhibits the growth of all bacterial strains under the test conditions at a concentration of 3.0%. Depending on the specific organism, complete inhibition can occur at a concentration as low as 1.0%.

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***Escherichia coli* K-12**

Sample	Alka-Hydroxy® Percent Concentration					
	0.0%	0.5%	1.0%	2.0%	3.0%	5.0%
1	0.940	0.492	0.000	0.000	-0.001	-0.001
2	0.944	0.489	0.000	0.000	-0.001	0.000
3	0.934	0.481	0.000	0.000	-0.001	-0.001
4	0.984	0.501	0.000	0.000	0.000	0.000
5	0.971	0.494	0.001	0.000	0.000	0.001
6	1.000	0.490	0.001	0.000	0.000	0.006
AVERAGE	0.962	0.491	0.000	0.000	-0.001	0.001
STD DEV	0.027	0.007	0.001	0.000	0.001	0.003
% Inhibition	0.0%	49.0%	100.0%	100.0%	100.0%	100.0%

***Escherichia coli* O157:H7**

Sample	Alka-Hydroxy® Percent Concentration					
	0.0%	0.5%	1.0%	2.0%	3.0%	5.0%
1	0.387	0.205	0.000	0.000	-0.001	-0.001
2	0.359	0.203	0.000	-0.001	-0.001	0.000
3	0.370	0.196	0.001	0.000	0.000	0.001
4	0.365	0.200	-0.001	-0.001	-0.002	-0.001
5	0.368	0.200	-0.001	0.000	-0.001	0.001
6	0.345	0.219	0.000	0.001	0.000	0.000
AVERAGE	0.366	0.204	0.000	0.000	-0.001	0.000
STD DEV	0.014	0.008	0.001	0.001	0.001	0.001
% Inhibition	0.0%	44.3%	100.0%	100.0%	100.0%	100.0%

Salmonella enterica

Sample	Alka-Hydroxy® Percent Concentration					
	0.0%	0.5%	1.0%	2.0%	3.0%	5.0%
1	0.293	0.143	0.000	0.001	-0.001	0.001
2	0.301	0.143	-0.001	-0.001	-0.001	0.000
3	0.294	0.138	-0.001	0.000	0.000	0.001
4	0.278	0.145	-0.001	0.000	-0.001	0.001
5	0.274	0.144	-0.001	0.002	-0.001	0.001
6	0.261	0.151	-0.002	0.001	-0.001	0.002
AVERAGE	0.284	0.144	-0.001	0.001	-0.001	0.001
STD DEV	0.015	0.004	0.001	0.001	0.000	0.001
% Inhibition	0.0%	49.3%	100.0%	100.0%	100.0%	100.0%

Enterococcus faecalis

Sample	Alka-Hydroxy® Percent Concentration					
	0.0%	0.5%	1.0%	2.0%	3.0%	5.0%
1	0.262	0.267	0.058	0.020	0.000	-0.001
2	0.261	0.266	0.057	0.031	-0.001	-0.002
3	0.265	0.267	0.064	0.040	-0.001	-0.001
4	0.262	0.277	0.067	0.044	-0.001	-0.002
5	0.274	0.276	0.079	0.052	0.000	0.000
6	0.261	0.271	0.120	0.073	0.000	0.001
AVERAGE	0.264	0.271	0.074	0.043	-0.001	-0.001
STD DEV	0.005	0.005	0.024	0.018	0.001	0.001
% Inhibition	0.0%	0.0%	72.0%	83.7%	100.0%	100.0%

Staphylococcus aureus

Sample	Alka-Hydroxy® Percent Concentration					
	0.0%	0.5%	1.0%	2.0%	3.0%	5.0%
1	0.164	0.113	0.090	0.000	0.000	-0.001
2	0.172	0.114	0.053	0.000	-0.002	-0.002
3	0.178	0.105	0.099	0.001	-0.002	-0.003
4	0.196	0.109	0.090	0.002	-0.001	-0.003
5	0.222	0.117	0.091	0.004	-0.002	0.000
6	0.276	0.166	0.128	0.004	-0.002	-0.002
AVERAGE	0.201	0.121	0.092	0.002	-0.002	-0.002
STD DEV	0.042	0.023	0.024	0.002	0.001	0.001
% Inhibition	0.0%	39.8%	54.2%	99.0%	100.0%	100.0%

Streptococcus pyogenes

Sample	Alka-Hydroxy® Percent Concentration					
	0.0%	0.5%	1.0%	2.0%	3.0%	5.0%
1	0.123	0.004	0.001	0.000	-0.006	-0.006
2	0.142	0.003	0.003	0.007	-0.009	-0.007
3	0.123	0.005	0.004	0.013	-0.004	-0.011
4	0.146	0.002	0.006	0.016	-0.003	-0.010
5	0.128	0.006	0.010	0.028	-0.002	-0.008
6	0.104	0.007	0.010	0.030	-0.003	-0.003
AVERAGE	0.128	0.005	0.006	0.016	-0.005	-0.008
STD DEV	0.015	0.002	0.004	0.012	0.003	0.003
% Inhibition	0.0%	96.1%	95.3%	87.5%	100.0%	100.0%





