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To: Jim Dooley

From: Mother Earth Minerals

(Five Pages)

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A FOOD TESTING LABORATORY SINCE 1967

PROJECT REPORT
RESEARCH MICROBIOLOGY DEPARTMENT

DATE: August 29, 2006

PREPARED FOR:

CLIENT CONTACT:

TITLE: MIC and MBC Evaluation of Pure Products Antimicrobial Product against Selected Bacteria.

OBJECTIVE: To determine the MIC (minimal inhibitory concentration) and MBC (minimal bactericidal concentration (MBC) for Pure Products antimicrobial product against *E. coli* and *Staphylococcus aureus* using standard NCCLS/CLSI methods.

EXPERIMENTAL APPROACH:

A. TEST PRODUCT AND MATERIALS

The client provided sufficient Pure Products antimicrobial product to use in the study.

B. TEST MICROORGANISMS

Generic *Escherichia coli* (ATCC #11229) and methicillin resistant *Staphylococcus aureus* (ATCC #700698) were used in the MIC and MBC evaluations. Generic *E. coli* is a common Gram-negative bacterial indicator of human/animal feces in food and water. *S. aureus* is a common Gram-positive bacterial pathogen associated with clinical (e.g., skin and nosocomial infections). Antibiotic resistant strains of *S. aureus* are of particular clinical concern.

C. MIC AND MBC PROCEDURES

MIC (minimal inhibitory concentration) assays for the antimicrobial were conducted separately against each selected microorganism (see section B) using the broth macro-dilution procedure for antibiotic susceptibility testing per NCCLS/CLSI methods ("National Committee for Clinical Laboratory Standards"; now referred to as "Clinical and Laboratory Standards Institute"). The inoculum for each microorganism was prepared and the MIC assay conducted according to respective culture methods described by NCCLS (M7-A5, 2000).

The *E. coli* and *S. aureus* cultures were purified and cultured using pre-poured Tryptic Soy agar (TSA) plates (incubated for 20-24 h at 35°C). Bacterial suspensions were each prepared from the respective TSA plates and diluted per NCCLS method (to a 0.5 McFarland standard

AquaVivos (dba Pure Products)
Its Effectiveness
Against E.Coli

Recent tests were conducted to determine the effectiveness of AquaVivos (dba Pure Products) against Escherichia coli (E. coli) bacteria.
The tests were conducted over a 24 hour period by ABC Research Corporation, a food testing laboratory, in Gainesville, FL.

Table 2. Minimal Bactericidal Concentration (MBC) Results for *E. coli*.

Antimicrobial Dilution		Bacterial Counts	
		CFU/ml	Log_{10} CFU/ml
	Initial Inoculum	590,000	5.77
1:6	Survivor Count	<10	<1.00
	Log_{10} Reduction		>4.77
	% Reduction		>99.998
1:12	Survivor Count	<10	<1.00
	Log_{10} Reduction		>4.77
	% Reduction		>99.998
1:24	Survivor Count	<10	<1.00
	Log_{10} Reduction		>4.77
	% Reduction		>99.998
1:48	Survivor Count	<10	<1.00
	Log_{10} Reduction		>4.77
	% Reduction		>99.998
1:96	Survivor Count	1,200	3.08
	Log_{10} Reduction		2.69
	% Reduction		99.8

Notes: 1) Counts expressed as CFU (colony forming units/ml).
2) Log Reduction (log count of initial inoculum) - (log count of subject survivors).
3) The MBC (i.e., at least a 3-log or 99.9% reduction) was at a 1:48 dilution of the antimicrobial.

The dilution rate of 1:48 (1 part AquaVivos to 48 parts water) provided the most efficient results, reducing the E. coli bacteria 99.998%.

in sterile 0.85% saline) to obtain respective cell suspensions to inoculate the macro-dilution series. The final inoculum suspensions were enumerated by making appropriate serial dilutions in Butterfield's phosphate buffer (BPB) and spread plating 0.1 ml of each dilution onto pre-poured TSA plates. The corresponding MIC assays were conducted using Mueller Hinton broth (MHB) to prepare the macro-dilution series of the antimicrobial product (i.e., from 1:6 through 1:24576) in MHB. The (MIC) dilution tubes were incubated for 24 h at 35°F. The MIC is the lowest concentration of antimicrobial agent that completely inhibits growth of the bacteria.

Minimal bactericidal concentration (MBC) assays were determined concurrently against each microorganism per standard methods (i.e., Manual of Clinical Microbiology, 2003). After incubation of the MHB tubes and determination of the MIC, the surviving bacteria in each tube with concentrations at and above the MIC was enumerated in duplicate using spread plating technique and pre-poured TSA plates. Using the initial inoculum counts and the viable counts in respective MHB tubes, the lethality of each respective tube was determined. The MBC is the lowest concentration of antimicrobial which yields at least 99.9% reduction (i.e., 3 log-units) during the MIC incubation (i.e., 24 h at 35°C).

RESULTS:

The minimal inhibitory concentration (MIC) results for the Pure Product antimicrobial against *E. coli* and *Staphylococcus aureus* are presented in Table 1. The MIC value for the antimicrobial against *E. coli* was at the 1:48 dilution (i.e., 2.08% concentration of active agent) and that for the *S. aureus* at the 1:24 dilution (i.e., 4.17% concentration of active agent).

The minimal bactericidal concentration (MBC) results for the Pure Product antimicrobial against *E. coli* and *Staphylococcus aureus* are presented in Tables 2 and 3, respectively. Note that these bactericidal (reduction) results apply to a 24 h period in a growth medium per the standard NCCLS/CLSI methods. There was a greater than 4.77 $\log_{10}$-unit (>99.998%) reduction of *E. coli* at the antimicrobial's 1:48 dilution and a 3.08 $\log_{10}$-unit reduction (99.8%) at the 1:96 dilution. For *S. aureus*, there was a 2.11 $\log_{10}$-unit (99.2%) reduction at the 1:6 dilution, a 2.13 $\log_{10}$-unit (99.3%) reduction at the 1:12 dilution, and a 1.13 $\log_{10}$-unit reduction (92.6%) at the 1:24 dilution.

In summary, these results indicated that the Pure Product antimicrobial was more effective in the inhibition and reduction of *E. coli* (a Gram-negative bacterium) than of *S. aureus* (a Gram-positive bacterium).

PREPARED BY: _____

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Table 1. Minimal Inhibitory Concentration (MIC) Results for *E. coli* and *S. aureus*

Antimicrobial Dilution	*E. coli* (growth)	*S. aureus* (growth)
1:6	–	–
1:12	–	–
1:24	–	– (MIC)
1:48	– (MIC)	+
1:96	+	+
1:192	+	+
1:384	+	+
1:768	+	+
1:1536	+	+
1:3075	+	+
1:6144	+	+
1:12288	+	+
1:24576	+	+

Notes: 1) The MIC is the lowest concentration of antimicrobial agent that completely inhibits growth of the bacteria.

2) The MIC was at the 1:48 dilution of the antimicrobial for *E. coli* and the 1:24 dilution of the antimicrobial for *S. aureus*.

Table 2. Minimal Bactericidal Concentration (MBC) Results for *E. coli*.

Antimicrobial Dilution		Bacterial Counts	
		CFU/ml	Log_{10} CFU/ml
	Initial Inoculum	590,000	5.77
1:6	Survivor Count	<10	<1.00
	Log_{10} Reduction		>4.77
	% Reduction		>99.998
1:12	Survivor Count	<10	<1.00
	Log_{10} Reduction		>4.77
	% Reduction		>99.998
1:24	Survivor Count	<10	<1.00
	Log_{10} Reduction		>4.77
	% Reduction		>99.998
1:48	Survivor Count	<10	<1.00
	Log_{10} Reduction		>4.77
	% Reduction		>99.998
1:96	Survivor Count	1,200	3.08
	Log_{10} Reduction		2.69
	% Reduction		99.8

Notes: 1) Counts expressed as CFU (colony forming units/ml).
2) Log Reduction = (log count of initial inoculum) - (log count of subject survivors).
3) The MBC (i.e., at least a 3-log or 99.9% reduction) was at a 1:48 dilution of the antimicrobial.

Table 3. Minimal Bactericidal Concentration (MBC) Results for *S. aureus*.

Antimicrobial Dilution		Bacterial Counts	
		CFU/ml	Log_{10} CFU/ml
	Initial Inoculum	270,000	5.43
1:6	Survivor Count	2,100	3.32
	Log_{10} Reduction		2.11
	% Reduction		99.2
1:12	Survivor Count	2,000	3.30
	Log_{10} Reduction		2.13
	% Reduction		99.3
1:24	Survivor Count	20,000	4.30
	Log_{10} Reduction		1.13
	% Reduction		92.6

Notes: 1) Counts expressed as CFU (colony forming units/ml).
2) Log Reduction = (log count of initial inoculum) - (log count of subject survivors).
3) The MBC (i.e., at least a 3-log or 99.9% reduction) was not achieved at any dilution of the antimicrobial.