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Evaluation of Antibacterial and Toxicological Effects of a Novel Sodium Silicate Complex

[1]D.A. Vattem, [1]V. Maitin and [2]C.R. Richardson
[1]Nutrition Biomedicine and Biotechnology Laboratory, Texas State University, 601 University Drive, San Marcos, TX 78666
[2]Department of Agriculture, Texas State University, 601 University Drive, San Marcos, TX 78666

Corresponding Author: D.A. Vattem, Nutrition Biomedicine and Biotechnology Laboratory, Texas State University, 601 University Drive, San Marcos, TX 78666 Tel: 512-245-7655

ABSTRACT

The potential for a sodium silicate complex (SSC) to reduce Post-harvest contamination of foods and water was evaluated in antibacterial activity assays against four Gram positive, Gram negative and clinically isolated multidrug resistant strains of bacteria. SSC inhibited the growth of all bacterial strains and the Minimal inhibitory Concentration (MIC_{90}) was in the range of 21.3-26.6 μg mL^{-1} for Gram negative bacteria, 10.6-53.2 μg mL^{-1} for Gram positive bacteria and 42.5-212.7 μg mL^{-1} the antibiotic resistant strains. Additionally, the Lethal Dose (LD_{50}) for acute oral toxicity of SSC determined in Female Albino Sprague-Dawley rats by oral gavaging was found to be >5000 mg kg^{-1}, Results suggest potential pharmaceutical, food safety and water quality application of SSC and merits further investigation.

Key words: Sodium silicate, antibacterial activity, acute oral toxicity, minimal inhibitory concentration, lethal dose, multi drug resistant

INTRODUCTION

Elemental silicon (Si) and silicon dioxide (SiO_2) can react with oxides and hydroxides of alkaline metals at high temperatures to form a variety silicate species (Iler, 1979; Nordstrom *et al.*, 2011) differing in molecular weights, physical and electrochemical properties. These differences have been exploited for industrial applications as polymers, semiconductors, stabilizers in glass, cosmetic, electronics and petroleum industries (Jones and Handreck, 1967; Baehr and Koehl, 2007). Sodium metasilicate is an approved food additive and has been granted GRAS status by the FDA (21CFR 182.90). Importance of silicon on human health is unclear and nutritionally, it has been categorized as a trace mineral, important in bone, structural and connective tissue development (Nielsen, 1984; Watts *et al.*, 2003; Sahin *et al.*, 2006). Animals consuming silicon free diets have poor skeletal development and joint strength (Carlisle, 1970; Carlisle, 1972; Katouli *et al.*, 2010). Silicates are essential for plant growth and external application of silicates has shown to improve yields and reduce fungal diseases (Belanger *et al.*, 1995; Li *et al.*, 2009; Ashokkumar *et al.*, 2011). However, the effects of silicates on bacterial diseases contamination and spoilage have not been investigated thoroughly (Rahim *et al.*, 1999). We have recently reported that a novel sodium silicate complex ($Na_{8.2}Si_{4.4}H_{9.7}O_{17.6}$; MW 563.4 mol^{-1}) has antioxidant (Townsend *et al.*, 2010a) and antiretroviral properties (Townsend *et al.*, 2010b). In anti-pathogenic/antivirulence assays, sub-

lethal levels this Sodium Silicate Complex (SSC) were effective in changing the composition of the *Pseudomonas aeruginosa* exopolysaccharide (EPS) monomers and may affect the adherence of this opportunistic pathogen to different matrices (Townsend *et al*., 2010b). To determine the potential of this SSC in reducing post-harvest microbial contamination of food and water, we evaluated antibacterial effect against Gram-negative, Gram-positive and clinically isolated strains of pathogens. Acute oral toxicity of SSC was also determined in female albino Sprague-Dawley rats by oral gavaging.

MATERIALS AND METHODS

Study compound: Sodium silicate complex ($Na_{8.2}Si_{4.4}H_{9.7}O_{17.6}$ M.Wt. 563.4 mol^{-1}) manufactured using a pyrosynthesis reaction was supplied by Cisne Enterprises Inc. (Odessa, TX). The total concentration of silicates (212.73 mg mL^{-1}) were quantified by the ammonium molybdate assay at 450 nm described previously (Townsend *et al*., 2010a).

Bacterial growth conditions: Gram-negative bacteria *E. coli* K-12 (ATCC 700926), *E. coli* O157:H7 (ATCC 25922) *E. coli* O157:H7 (ATCC 35150) and *Salmonella enterica* serovar Typhimurium LT2 (ATCC 15277) and Gram-positive bacteria were *Enterococcus faecalis* (ATCC 19433), *Staphylococcus aureus* (ATCC 12600), *S. aureus* (ATCC 25923) and *Streptococcus pyogenes* (ATCC 19615) were purchased from American Type Culture Collection (ATCC, Rockville, MD, USA). Antibiotic resistant strain of *E. coli*, *S. aureus*, *Pseudomonas aeruginosa* and *Enterobacter cloacae fluorescens* (Table 1) isolated from clinical subjects were a kind gift of Dr. Irene Lopez Lozoya (International Laboratory References and Services, Torreon, Mexico). Recommended media and growth conditions (Table 2) were used to prepare stock cultures in dimethyl sulfoxide and stored at -80°C.

Antibacterial activity and MIC_{90}: A broth microdilution method for susceptibility testing of antibacterial agents as recommended by the Clinical and Laboratory Standards Institute was used

Table 1: Antibiotic resistance in bacterial strains isolated from clinical subjects

	Bacterial strains isolated from clinical subjects			
	E. coli	*S. aureus*	*P. aeruginosa*	*E. cloacae*
Antibiotics	MIC_{90} Concentration (μg)			
Amikacin	30		30	30
Carbenicillin			100	
Cefotaxime	30		30	30
Cephalothin		30		
Chloramphenicol	30			30
Ciprofloxacin	5	5	5	
Dicloxacillin		1		
Erythromycin		15		
Gentamicin	10	10	10	10
Netromycin	30			30
Nitrofurantoin	300		300	30
Penicillin		10		
Sulfamethoxazole-trimethoprim	25	25		
Vancomycin		30		

Table 2: Standard media and growth conditions used for different bacterial strains tested

Strain	Media	Growth conditions (°C)
Enterococcus faecalis (ATCC 19433)	Brain heart infusion broth	37
Escherichia cloacae (Clinical Isolate)	Nutrient broth	37
Escherichia coli (ATCC 25922)	Trypticase soy agar	37
Escherichia coli (Clinical Isolate)	Trypticase soy agar	37
Escherichia coli K-12 (ATCC 700926)	M9 minimal medium	37
Escherichia coli O157:H7 (ATCC 35150)	Trypticase soy agar	37
Pseudomonas aeruginosa (Clinical Isolate)	Trypticase soy agar	37
Salmonella enterica (ATCC 15277)	Nutrient broth (0.5% NaCl)	37
Staphylococcus aureus (ATCC 12600)	Nutrient broth	37
Staphylococcus aureus (ATCC 25923)	Trypticase soy agar	37
Staphylococcus aureus (Clinical Isolate)	Trypticase soy agar	37
Streptococcus pyogenes (ATCC 19615)	Nutrient broth (0.5% NaCl)	37

to determine the antimicrobial activity of SSC (Mansouri *et al*., 2011; Iroha *et al*., 2011). Briefly, 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 10^8 colony-forming units (CFU) mL^{-1}. To improve cell yield in the *Enterococcus faecalis* and *Streptococcus pyogenes* cultures, the Cation-adjusted Mueller-Hinton broth was supplemented with 2.5 mg mL^{-1} of yeast extract (Becton-Dickinson). Appropriate dilutions of SSC were prepared in sterile Cation-adjusted Mueller-Hinton broth such that the final concentrations of the product were 0.0 (Control) 3.2, 6.6l, 10.6, 13.2, 21.3, 26.6, 42.5, 53.2, 63.8, 106.4 and 212.7 µg mL^{-1}. Appropriate volumes of the standardized cell suspension were added to the dilutions of the product to achieve a final cell density of 5×10^5 CFU mL^{-1}. The dilutions were then aliquoted into the wells of a 96-well microtiter plate (with a volume of 0.2 mL^{-1} well) and incubated at 35°C for 16-20 h along with no cell controls. The culture turbidity (A_{590}) was measured by using a microtiter plate reader (Biotek, Winooski, VT) and used to calculate the Percent inhibition in growth using the following formula:

$$\text{Growth inhibition}(\%) = \left(\left[\frac{A_{590}^{\text{Control}} - A_{590}^{\text{silicate}}}{\left[A_{590}^{\text{Control}}\right]}\right]\right) \text{x}100$$

The concentration of the sodium silicate that inhibited the growth of bacteria by 90% or more was designated as MIC_{90}.

Determination of acute oral toxicity and LD_{50}: To determine potential suitability of sodium silicate complex in food safety applications, Acute oral toxicity potential of the sodium silicate complex was evaluated in female (nulliparous and non-pregnant) Sprague-Dawley albino rats (Vijayabalaji *et al*., 2010; Singh *et al*., 2012). The animal experiments were in accordance with Environmental protection agency health effects test guidelines (EPA, 2002) and conformed to Guide for the Care and Use of Laboratory Animals (NRC, 2011) and were approved by the Institutional Animal Care and Use Committee. Young, adult female (nulliparous and non-pregnant) Sprague-Dawley Albino rats with starting weight of 178-184 g were acclimated to laboratory conditions

(22±3°C; RH-30-70%, 12 h dark/light cycle) for 5 days. Rats without any abnormality or pathological change were used in the study (Wiam *et al.*, 2005). The animals were fed ad libitum with water and a commercial rodent diet Formulab # 5008 (PMI Feeds Inc. Saint Louis, MO) except for approximately 16 h before dosing. Three animals were randomly selected and an individual dose was calculated for each animal based on its fasted body weight and administered by gavage at a volume of 5.82 mL kg^{-1} (5209 mg kg^{-1}). Clinical/behavioral signs of toxicity were made at least three times on the day of dosing (day 0) and at least once thereafter got 14 days. Individual body weights were recorded prior to dosing on days 7 and 14. On day 14 after dosing each animal was euthanized by an overdose of CO_2, all animals were subjected to gross necropsy and all abnormalities were recorded.

Statistical analysis: For antibacterial assays, experiments were replicated twice for each concentration and a minimum of six replicates were set up for each concentration and used to calculate the average mean and standard deviation. Data were subjected to analysis of variance and differences between means were regarded to be statistically significant when $p<0.05$.

RESULTS

Antibacterial effect on gram negative bacteria: Sodium silicate complex was effective in inhibiting the growth in all Gram negative bacteria tested (Fig. 1). In the control strains, used to test the validity of the antibacterial assays, *E. coli* K-12 (ATCC 700926) and *E. coli* (ATCC 25922) the MIC_{90} was determined to be 21.3 and 26.6 μg mL^{-1}, respectively (Table 2). The MIC_{90} values for both *E. coli* O157:H7 (ATCC 35150) and *S. enterica* (ATCC 15277) were determined to be 21.3 μg mL^{-1} (Table 3).

Antibacterial effect on gram positive bacteria: Sodium silicate complex at 53.2 and 42.5 μg mL^{-1} inhibited the growth of *E. faecalis* (ATCC 19433) and *S. aureus* (ATCC 12600), respectively by more than 90% (Table 4). The MIC_{90} for another strain of *S. aureus* (ATCC 25923) was determined to be 26.6 μg mL^{-1} (Table 4). Among all Gram positive bacteria, the MIC_{90} for *S. pyogenes* (ATCC 19615) was the lowest and was determined to be 10.6 μg mL^{-1} (Table 4). Overall, sodium silicate was effective in inhibiting the growth in all Gram-positive bacteria tested (Fig. 2).

Table 3: MIC_{90} of SSC against tested Gram-negative bacterial strains

Strain	MIC_{90} (μg mL^{-1})
Escherichia coli (ATCC 25922)	26.6
Escherichia coli K-12 (ATCC 700926)	21.3
Escherichia coli O157:H7 (ATCC 35150)	21.3
Salmonella enterica (ATCC 15277)	21.3

Table 4: MIC_{90} of SSC against tested Gram-positive bacterial strains

Strain	MIC_{90} (μg mL^{-1})
Enterococcus faecalis (ATCC 19433)	53.2
Staphylococcus aureus (ATCC 12600)	42.5
Staphylococcus aureus (ATCC 25923)	26.6
Streptococcus pyogenes (ATCC 19615)	10.6

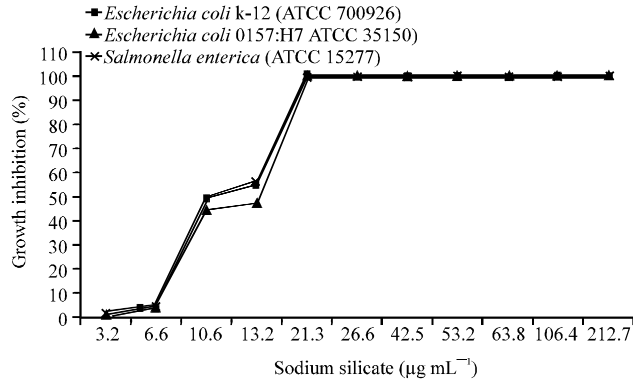

Fig. 1: Inhibitory effect of SSC against tested Gram-negative bacterial strains

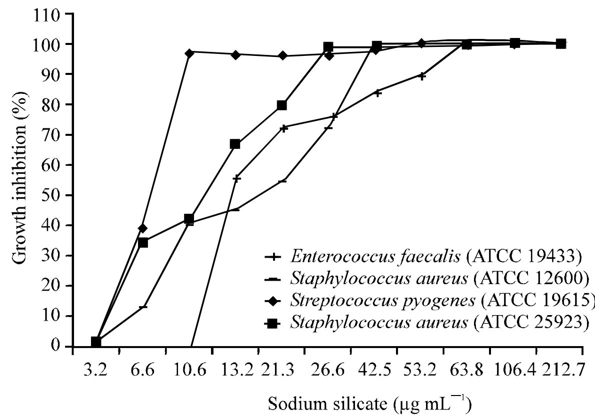

Fig. 2: Inhibitory effect of SSC against tested Gram-positive bacterial strains

Table 5: MIC_{90} of SSC against clinically isolated multi-drug resistant bacterial strains

Strain	MIC_{90} (µg mL^{-1})
Escherichia cloacae (clinical isolate)	212.7
Escherichia coli (clinical isolate)	42.5
Pseudomonas aeruginosa (clinical isolate)	42.5
Staphylococcus aureus (clinical isolate)	106.4

Antibacterial effect on antibiotic resistant bacteria: Sodium silicate complex inhibited the growth of all four strains of bacteria resistant to different antibiotics (Table 3) (Fig. 3). The MIC_{90} for *E. cloacae* (212.7 µg mL^{-1}) was highest for all the bacteria tested. The MIC_{90} for antibiotic resistant *S. aureus* was 106.4 µg mL^{-1} (Table 5) which was higher than the MIC_{90} for two other strains of *S. aureus* (Table 5). The MIC_{90} for the drug resistant strain of *E. coli* was also higher than the non-drug resistant strains and was determined to be 42.5 µg mL^{-1}. The complex was also effective in inhibiting the growth of multi-drug resistant *P. aeruginosa* and the MIC_{90} was calculated to be 42.5 µg mL^{-1} (Table 5).

Acute oral toxicity and LD_{50}: There was no mortality observed during the study. The body weight gain was not affected by the administration of sodium silicate complex (Table 6). All animals

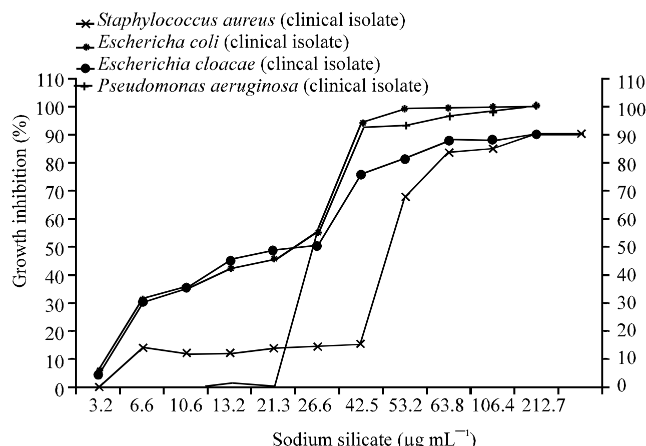

Fig. 3: Inhibitory effect of SSC against clinically isolated multi-drug resistant bacterial strains

Table 6: Evaluation of acute oral toxicity effect of SSC at 5209 mg kg^{-1} by oral-gavaging in female albino Sprague-Dawley rats

Body weight (g) (Days)				
0	7	14	Clinical signs/behavior	Gross necropsy findings
178	212	231	NOA	NOA
184	221	240	NOA	NOA
178	220	230	NOA	NOA

NOA: No observable effects

appeared normal for the duration of the study with no abnormal clinical signs or behavior (Table 6). The gross necropsy at the termination of the termination of the study revealed no observable abnormalities. The LD_{50} was determined to be greater than 5000 mg kg^{-1}.

CONCLUSIONS

SSC exhibited antimicrobial activity against many Gram positive and Gram negative bacteria. The MIC_{90} values ranged from 21.3-26.6 μg mL^{-1} for Gram negative bacteria and 10.6-53.2 μg mL^{-1} for Gram positive pathogens. In addition, SSC was also effective in inhibiting the growth of clinically isolated multi-drug resistant strains of bacteria (MIC_{90} range: 42.5-212.7 μg mL^{-1}). A LD_{50} for oral toxicity suggests that the SSC may have potential applications in reducing clinical infections and microbial contamination of food and water.

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