

Efficacy of Chitosan sanitizer against pathogenic bacteria *in vitro*

Report Submitted to Shield Nutraceuticals, Inc
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Materials and Methods

The chitosan and citric acid were received from Shield Nutraceuticals, Inc. The sanitizer was made by dissolving equal amounts of chitosan and citric acid into deionized water, according to the manufacturer's instruction. The 1% and 0.25% of the sanitizer concentrations were tested. Deionized water was used as a control. Test bacteria include *Salmonella* Newport strain JJPX, *Listeria monocytogenes* strain F6854, *Escherichia coli* O157:H7 strain SEA 13B88, *Enterobacter aerogenes* strain ATCC 13048 and *Staphylococcus aureus* strain ATCC 6538.

The overnight culture of the tested organism was diluted in Tryptic soy broth (TSB) to get a ca. 10^6 CFU/mL inoculum. Five milliliters of the 2% or 0.5% sanitizer was mixed with 5 ml of the bacterial inoculum to get a final sanitizer concentration at 1% or 0.25% in the mixture. The sanitizer and bacterial mixtures were incubated at 35°C for 24 hours. After the incubation, a serial of dilutions were made in 0.1% peptone water, and plated on Tryptic soy agar (TSA) for the enumeration of the surviving cells. The TSA plates were incubated at 35°C for 24 hours, and the colonies on the plates were counted.

Results

The survival of *S. Newport* after 24 h sanitizer treatments is shown in Table 1. Compared to 0 hour, the *Salmonella* population reduced to 1 CFU/mL in the 1% solution, while the population increase was observed in the 0.25% solution.

Table 1. Survival of *S. Newport* (CFU/mL) after sanitizer treatments

	population
Inoculum at 0 h	1.04×10^6
1% chitosan at 24 h	1
0.25% chitosan 24 h	2.90×10^8
Control at 24 h	1.75×10^9

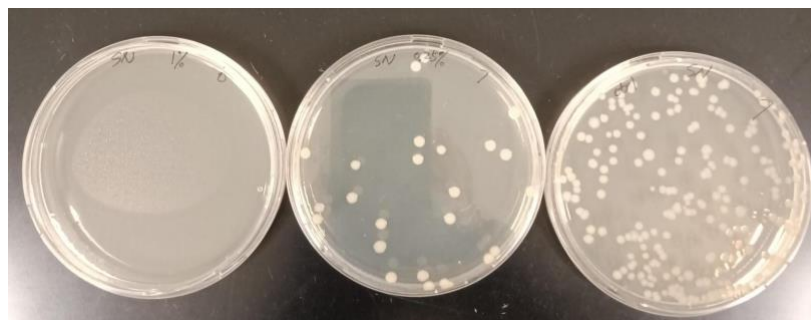


Figure 1. *S. Newport* colonies on TSA plates. Left: 1% chitosan; middle: 0.25% chitosan; right: control

The survival of *L. monocytogenes* after 24 h sanitizer treatments is shown in Table 2. Both the 1% and 0.25% sanitizers inhibited the growth of *L. monocytogenes*.

Table 2. Survival of *L. monocytogenes* (CFU/mL) after sanitizer treatments

	population
Inoculum at 0 h	1.47×10^6
1% chitosan at 24 h	3.60×10^2
0.25% chitosan 24 h	1.04×10^6
Control at 24 h	2.18×10^9

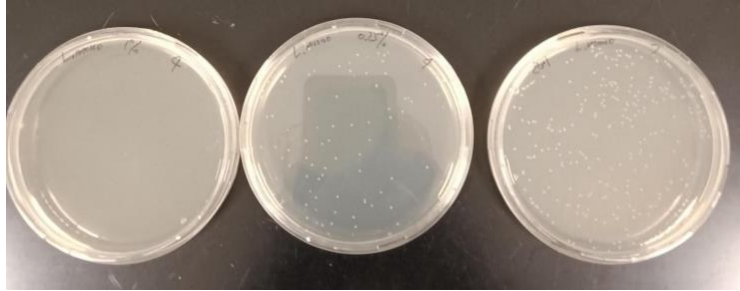


Figure 2. *L. monocytogenes* colonies on TSA plates. Left: 1% chitosan; middle: 0.25% chitosan; right: control

The survival of *E.coli* O157:H7 after 24 h sanitizer treatments is shown in Table 3. The 1% sanitizer reduced the *E.coli* O157:H7 population to below the detection limit (1 CFU/mL), and the 0.25% sanitizer reduced ca. 2 log reductions.

Table 3. Survival of *E.coli* O157:H7 (CFU/mL) after sanitizer treatments

	population
Inoculum at 0 h	1.64×10^6
1% chitosan at 24 h	<1
0.25% chitosan 24 h	3.75×10^4
Control at 24 h	5.95×10^8

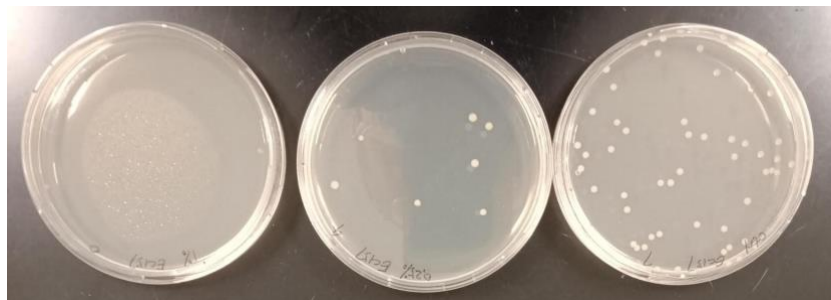


Figure 3. *E.coli* O157:H7 colonies on TSA plates. Left: 1% chitosan; middle: 0.25% chitosan; right: control

The survival of *E. aerogenes* after 24 h sanitizer treatments is shown in Table 4. The 1% sanitizer reduced the *E. aerogenes* population to below the detection limit (1 CFU/mL), while the 0.25% sanitizer did not inhibit the growth of the *E. aerogenes*.

Table 4. Survival of *E. aerogenes* (CFU/mL) after sanitizer treatments

	population
Inoculum at 0 h	1.24×10^6
1% chitosan at 24 h	<1
0.25% chitosan 24 h	3.55×10^7
Control at 24 h	6.80×10^8

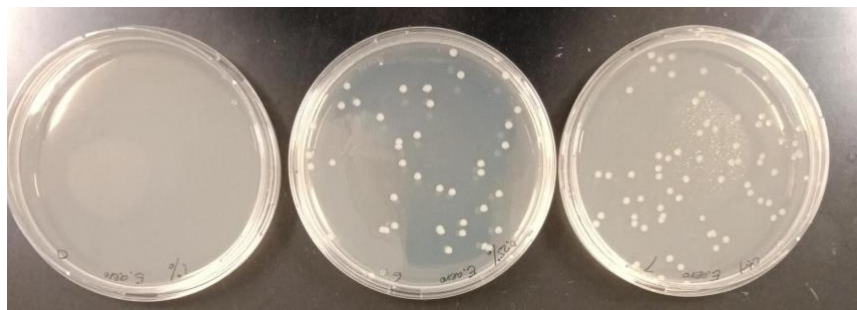


Figure 4. *E. aerogenes* colonies on TSA plates. Left: 1% chitosan; middle: 0.25% chitosan; right: control

The survival of *S. aureus* after 24 h sanitizer treatments is shown in Table 5. The 1% sanitizer reduced the *S. aureus* population to below the detection limit (1 CFU/mL), and the 0.25% sanitizer also reduced ca. 2 log reductions.

Table 5. Survival of *S. aureus* (CFU/mL) after sanitizer treatments

	population
Inoculum at 0 h	1.29×10^6
1% chitosan at 24 h	<1
0.25% chitosan 24 h	1.42×10^4
Control at 24 h	1.18×10^9

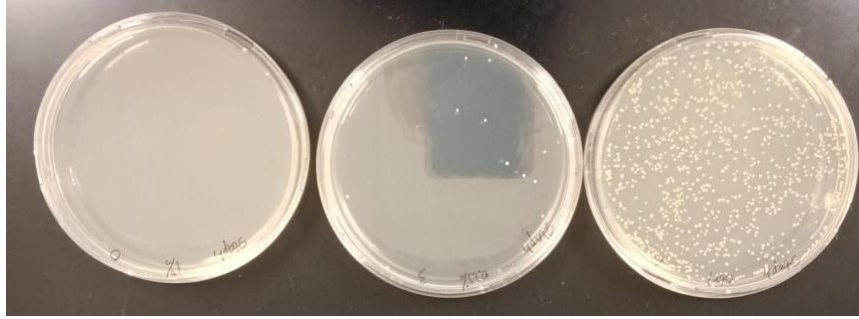


Figure 5. *S. aureus* colonies on TSA plates. Left: 1% chitosan; middle: 0.25% chitosan; right: control

Conclusions

The 1% chitosan sanitizer inhibited all 5 tested bacteria in varying degrees, including *S* Newport strain JJPX, *L. monocytogenes* strain F6854, *E. coli* O157:H7 strain SEA 13B88, *E. aerogenes* strain ATCC 13048 and *S. aureus* strain ATCC 6538. The 0.25% sanitizer inhibited *L. monocytogenes* F6854, *E. coli* O157:H7 SEA 13B88, and *S. aureus* ATCC 6538, but to a lesser degree compared to the 1% sanitizer.

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