RESEARCH IN PROCESS

Application of chitosan microparticles to eliminate foodborne pathogens in agricultural water that contacts fresh produce

SUMMARY

The consumption of raw fruits and vegetables requires prevention of contamination by foodborne pathogens in the pre- and post-harvest stages. Agricultural water, either of irrigation or produce sanitation, is a possible source of foodborne diseases (Figure 1). Disinfection of irrigation or wash water is most often carried out by treatment with chlorine. However, because treatment with chlorine results in only a 100-fold reduction and the creation of toxic byproducts, a safer, more effective, and less toxic treatment is needed. We are investigating the feasibility of using chitosan microparticles (CM, a cross-linked derivative of chitosan) as an alternative treatment for surface waters used for irrigation. Applications could also include introducing chitosan as a antimicrobial film on produce (Figure 2). Prior research showed that CM treatment required a concentration of 0.3% CM (w/v) to effectively reduce *Salmonella* inocula (100,000 CFU/ml) to below detectable levels in 24 h, and was not effective for stationary growth phase cells. CM efficacy was greatly reduced when used in conjunction with intact pond water. The current research aims to develop and optimize applications for treatment of agricultural water.

OBJECTIVES

1. Investigate practical pre-harvest application of chitosan microparticles (CM) for pathogen reduction in the complex medium of irrigation pond water.
2. Optimize use of CM films for post-harvest application to reduce/prevent contamination of pathogens on produce.
3. Conduct preliminary experiments to investigate CM activity against norovirus.
4. Assess cost-effectiveness of chitosan application to agricultural water.

RESULTS TO DATE

Thus far, CM was confirmed as an effective treatment for elimination of stationary phase *Salmonella* in pond water. Lowering the inocula by 10-fold resulted in non-detectable levels of *Salmonella* in pond water after just 24 hours of treatment with 0.1% CM (Figure 3). CM was effective with stationary cells in pond water, but activity was diminished in sterile deionized water (DI) water, which is contrary to a previous report. We hypothesize that unidentified predation or toxicity may work synergistically with CM. The effect of CM was not diminished over time, as CM that was aged in pond water for 24 hours prior showed similar efficacy to freshly prepared CM.

Future studies will examine different formulations of CM, combined application of CM with chlorine, and application to ponds with varying parameters of quality, and will further assess stability of CM. Finally, we will address the cost effectiveness of using CM as an alternative to chlorine or other sanitizers.

BENEFITS TO THE INDUSTRY

Chitosan has already been found to be useful for a wide range of applications. A limitation of chitosan is that, like chlorine, its effectiveness is greatly affected by pH. Chitosan microparticles, however, have been shown to have antimicrobial activity at neutral pH. An additional advantage over current treatment methods is that CM is a safe, biodegradable particle. Should CM prove to be a practical, cost-effective alternative to chlorine treatment, we could lower the incidence of outbreaks with foodborne pathogens without producing the toxic byproducts that arise from treatment with chlorine.

METHODS

Chitosan microparticles (CM) were prepared using a previously described laboratory method (Fan et al., 2017). Concentrations of CM ranging from 0.05 to 0.3% were prepared in either sterile distilled water (DI) or intact pond water. An overnight culture (stationary phase) of *Salmonella* Typhimurium ATCC 14028 (~10^9 CFU/mL) was washed and used to inoculate (10^4 CFU/mL) both treated and untreated DI or pond water samples in 250-mL Erlenmeyer flasks containing 100 mL of water. Flasks were incubated with shaking at 200 rpm and 37°C. At appropriate time points, survival of inoculated *Salmonella* was determined by plate count onto XLD agar.

Figure 1. Collecting pond water samples

Figure 2. CM film on tomato: post-harvest application

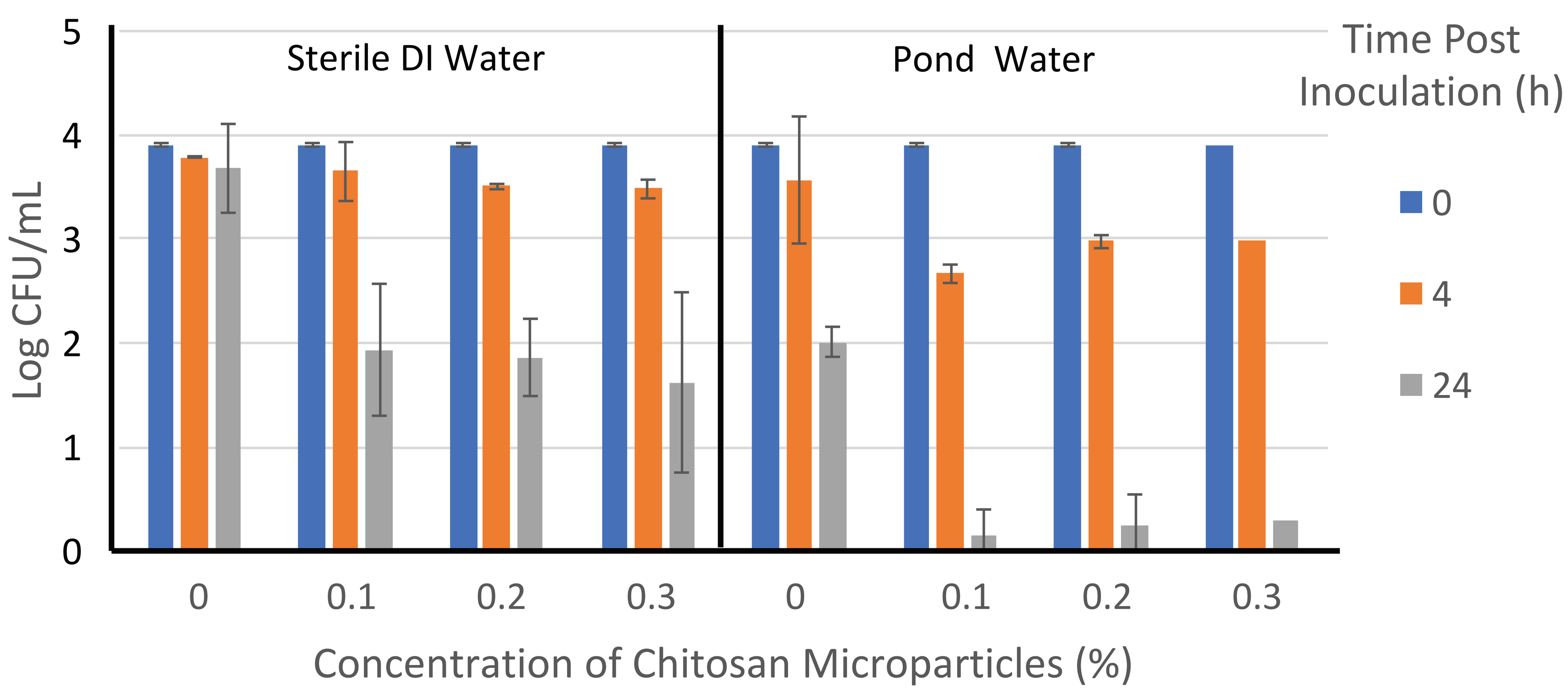

Figure 3. Effect of chitosan microparticles on survival of *Salmonella*

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