Antimicrobial activity of copper alone or in combination with ascorbic acid against *E. coli* O157:H7, *Salmonella* and *Cronobacter* spp. in laboratory medium

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Abstract

The aim of this study was to determine the effect of copper (50 ppm) alone or in combination of ascorbic acid (0.2%) on the survival and growth of *E .coli* O157:H7, *Salmonella* and *Cronobacter sakazakii* in laboratory medium (BHI broth). Bacterial growth was monitored during the incubation period for 8h at 37° C. Copper (50 ppm) or ascorbic acid (0.2%) alone slightly retarded the growth of all three tested bacteria. However, the growth of bacteria was significantly inhibited when Cu 50 ppm in combination with ascorbic acid 0.2% were added into BHI broth. These findings indicated that ascorbic acid in combination with copper sulfate, could be used as antimicrobial agent for the growth of pathogens.

Introduction

Eschericia Coli O157:H7, *Salmonella* spp. and *Cronobacter* spp. are all pathogens that are most commonly known for their harmful effects on food and the people who consume such contaminated foods (Meng & Doyle, 1998). *E. coli* O157:H7 is found mainly in meats such as beef, raw milk, vegetables as well as dairy products (Hussain, 2007). *Salmonella* is commonly found in unpasteurized milk, poultry and egg products(Rodrigue et al. 1990). *Cronobacter sakazaki* is an emerging human pathogen that contaminates powdered infant formula (PIF) and has been associated with various cases of neonatal meningitis (Kim & Loessner 2008; Jang & Rhee, 2009). All of these bacteria can, and have caused death. However, these cases may be avoided with proper sterilization and preparation of food. With prior knowledge of the effects of copper and ascorbic acids on bacteria (Ibrahim et al. 2008; Tajkarimi et al. 2010), we developed an experiment that would further our studies and help our understanding of the matter. In the past numerous studies have been done using various treatments and measuring the impact they may have on the population growth and reductions of such bacteria. In our study, treatments such as copper (50 ppm), ascorbic acid (0.2%) and a combination of both were used. We used optical density readings and plate count methods to test our theory on which treatments would effectively reduce the bacterial population of all three bacterial populations. Our goal is to drastically reduce the bacterial level. Using a control, a broth with no treatment, as our reference, we compared the optical density of each of our bacteria and their treatments. We predicted the combination of both copper and ascorbic acid would have the greatest reduction in population than any of the other treatments.

Objective

❖ To investigate the effect of lactic acid, acetic acid and copper (Cu) alone or in combination with ascorbic acid (Asc) against *E. coli* O157:H7 (43895), *Salmonella* Typhimurium ED405, and *Cronobacter sakazakii* 701 in laboratory media.

Methods

Experimental chart

E. Coli O157:H7 , *Salmonella, and Cronobacter sakazakii* strains

↓

Individual Strains + BHI broth(12 hrs,37 ºC)

↓

Wash cells in peptone water (centrifuge 700 rpm, 10 min, 4 ° C)

↓

Adjusted culture concentration approximately 3.00 log CFU/ml

↓

Inoculate 100 µl into treatments

↓

Vortex and incubate at 37 ºC for 8 hr

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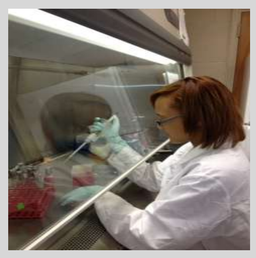
O.D. readings (610 nm) and plating

Cells were harvested by centrifugation



Sample inoculation

Sample incubation at 37 ºC

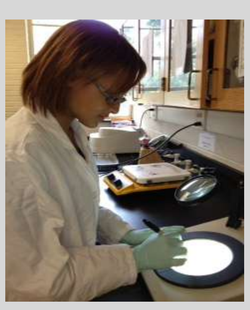
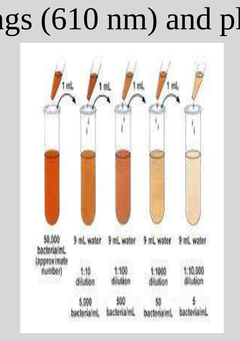
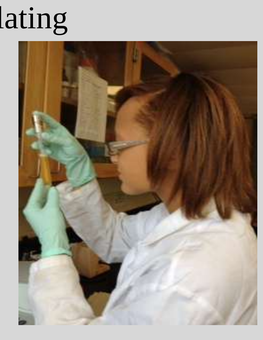


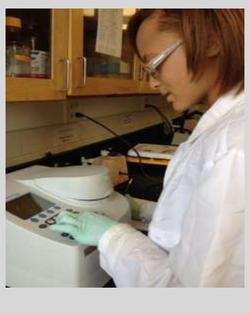
Plate count to determine the bacterial population



Serial dilutions and plating



Turbidity observed



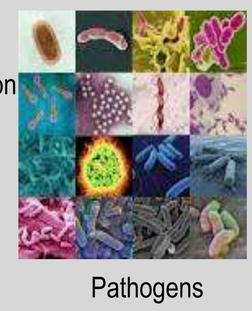
O. D. readings using spectrophotometer

Chemicals used

Media preparation

Treatment solution (Filter sterilization)

Antimicrobial effect on



Pathogens

Results

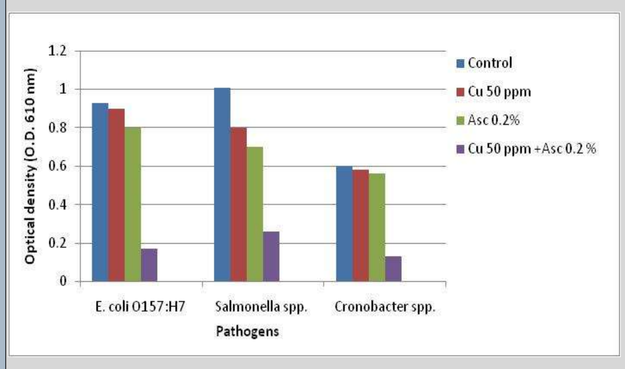


Fig. 1. Survival and growth of bacteria in BHI broth in the presence of copper (Cu) and ascorbic acid (Asc) after incubation at 37 ºC for 8 hr.

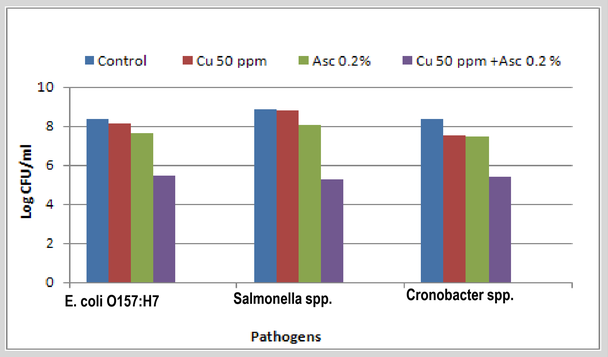


Fig. 2. Bacterial population (Log CFU/ml) in the presence of copper (Cu) and ascorbic acid (Asc).

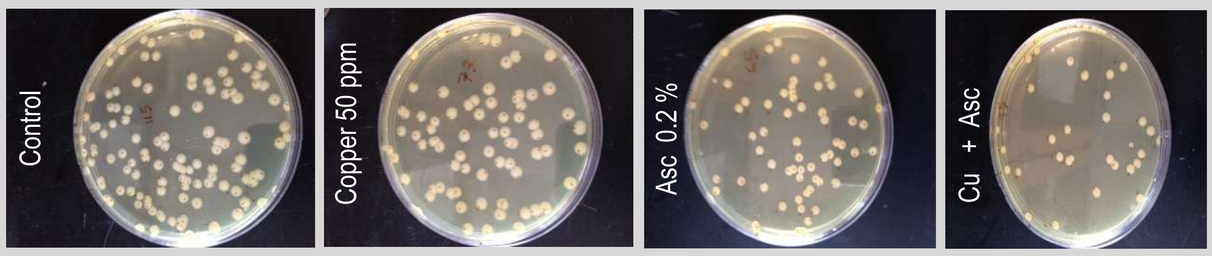


Fig. 3. Agar plates with different level of bacterial population showing effect of treatment (*E. coli* O157:H7)

Conclusions

❖This study demonstrates the ability of copper alone or in combination with lactic and acetic acid to inhibit the growth of tested foodborne pathogens.

❖Our finding shows that combination of copper and ascorbic acid could be a good alternative to control these pathogens.

❖Populations of *E. coli* O157:H7, *Salmonella* spp. and *Cronobacter sakazakii* reduced by 2.92, 3.59, and 3.0 log CFU/ml respectively when compared to their control population.

❖Further studies are needed to evaluate the effect of these treatments in food model including juice and infant formula.

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