

Bacterial Isolation & Efficacy of Natural Remedies for Bovine Mastitis

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Abstract

This study seeks to evaluate the preventative ability of various natural substances when exposed to isolated Mastitis-causing bacterial species. Bacteria were isolated from samples found at the university dairy. Using serial dilution and Kirby Bauer methods, natural remedies were applied to a plate prior to bacterial growth in order to evaluate their inhibition zones. It was found that apple cider vinegar (ACV) provided the greatest bactericidal effect for each species of bacteria isolated in this study. Tea tree oil and turmeric showed a few cases of prevention, while garlic and yogurt contaminated the plates and showed little to no prevention in majority of trials. It can be concluded that ACV has the strongest potential to be utilized as a preventative measure for dairy cattle Mastitis if a safe topical application is studied and tested in the future.

Introduction

Mastitis involves inflammation and infection of the mammary gland, and it is the most common disease in dairy cattle. Presence of this disease in dairy cattle leads to decreased milk production, poor milk quality, and therefore a decreased profit for the producer. The goal in this experiment is to determine the efficacy of utilizing natural resources to prevent Mastitis-causing bacteria growth. The bactericidal ability of each of substance tested has been previously studied, and they were found to provide resistance and treatment to Staphylococcus aureus and Escherichia coli. Specifically, the treatments being studied include apple cider vinegar (Yagnik et al. 2021), tea tree oil (Kwieciński et al. 2009), turmeric (Nurhayani and Avianto 2022), garlic (Abiy and Asefaw 2016) and yogurt (Attaie et al. 1987). Determining which bacteria are present at Del Val's dairy farm and testing the efficacy of these treatments for the isolated strains will provide helpful information for the microbiology field as well as for dairy herd management.

References

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Methods

- (1) Acquire milk samples and swab them on AccuMast plates for 24-hour incubation. This will isolate the bacteria based on species: *Streptococcus* spp., *Staphylococcus* spp., & Gram-Negative spp.
- (2) Obtain a single sample from the three quadrants on the plates and label. Utilizing serial dilution, create concentrations of each bacteria species in saline to transfer to Kirby Bauer plates.
- (3) Create concentrated samples of each natural remedy (plain yogurt, garlic, tea tree oil, apple cider vinegar, and turmeric) and soak the Kirby Bauer disks in each. Apply these to specific areas on the plates. Allow to incubate for another 24-48 hours. The bacteria will be growing during this time.
- (4) Observe the plates and measure the zones of inhibition (mm), to determine how well the samples prevented bacterial growth for each strain. Compare the values between the substances and between the bacterial strains to see which treatments are successful for each given case.

Results

Bacterial isolation and identification showed that various species were present in the DVU dairy samples: various *Streptococcus* spp., various *Staphylococcus* spp (chromogenes & haemolyticus), and Gram-Negative species: *Klebsiella* spp., & *Enterobacter* spp.

Streptococcus spp.

Apple cider vinegar (ACV) showed strong average inhibition (3mm), with tea tree oil (TTO) following behind (0.75mm).

Gram-Negative spp.

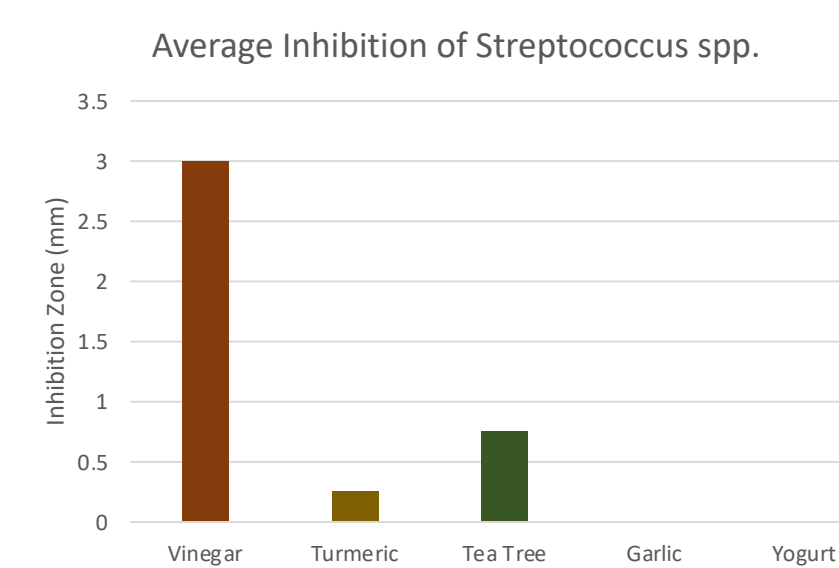
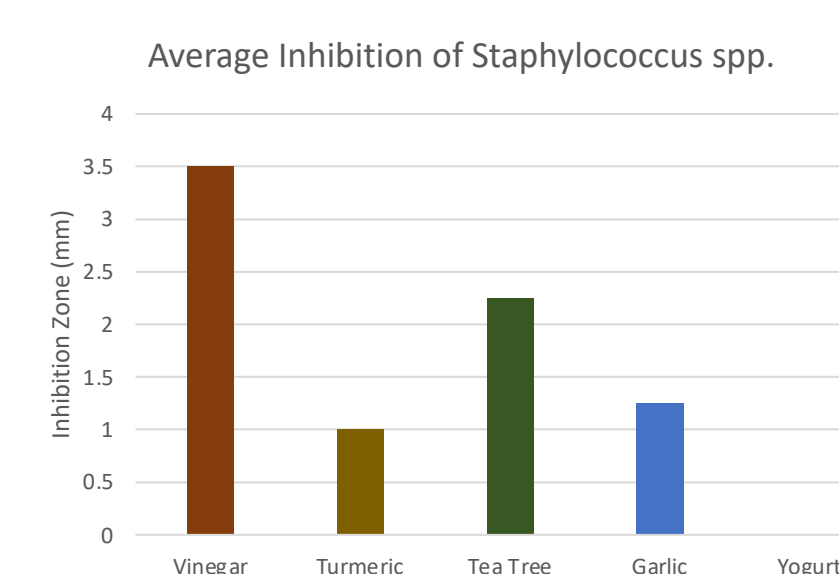
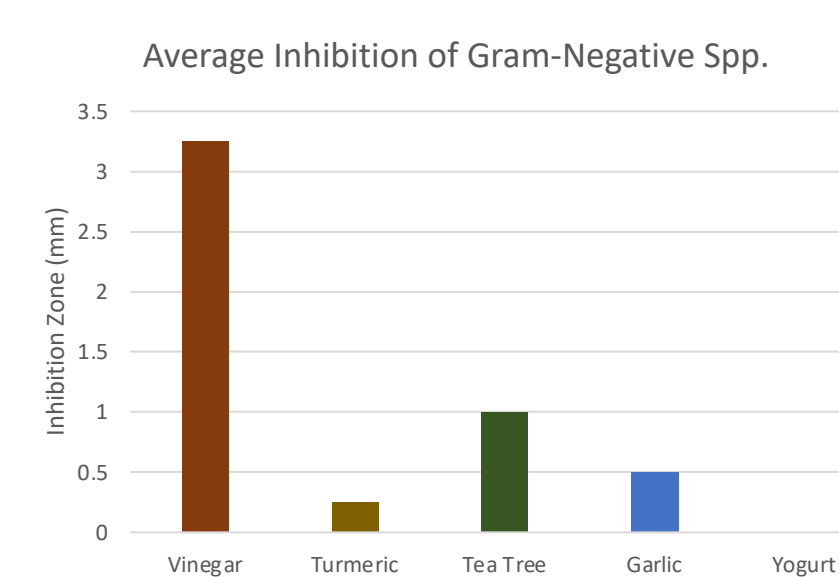
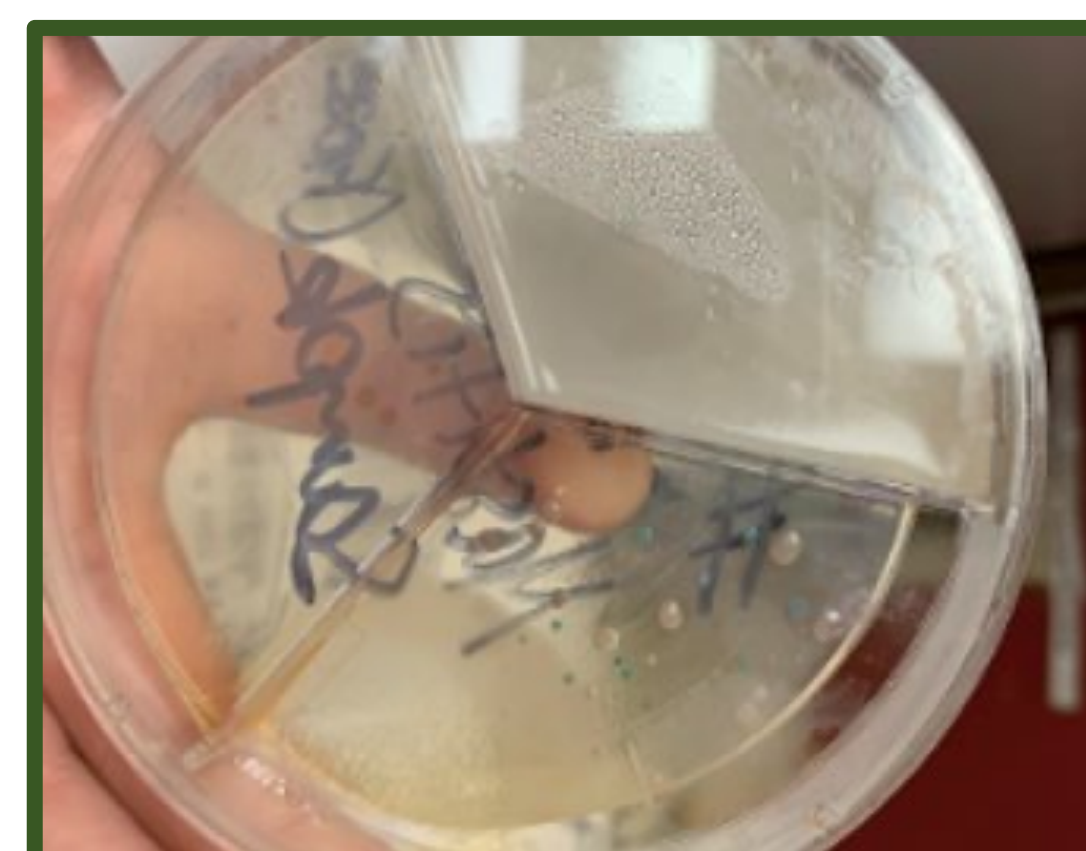
ACV inhibited 3.25mm and TTO at 1mm

Staphylococcus spp.

ACV inhibited at 3.5mm and TTO at 2.25mm

Turmeric inhibited growth on a few plates at low levels.

Yogurt and Garlic did not show inhibition in most cases and even promoted extra bacterial growth on the plates.



Discussion

It was expected that *E. coli* and *S. aureus* were going to be present in the Mastitis samples, as these are the most common causative bacterial agents for this infection. In this study, however, these two bacterial strains were not isolated from the given samples. However, the natural remedies that were previously found to prevent and treat against those two bacterial species also showed some preventative ability for the various species isolated in this study. Apple cider vinegar (ACV) had the greatest impairment on bacterial growth seen throughout nearly all of the samples. Compared to the other substances used, ACV showed a greater average inhibition zone for the *Streptococcus* spp. ($p=0.18$), *Staphylococcus* spp. ($p=0.27$), and the Gram-Negative spp. ($p=0.14$). Though the numbers are not fully statistically significant, it is likely that with additional cases and time to conduct the study, the values would show the same trend and become significant. Additional studies can be conducted with each bacterial species to determine the ideal concentration of ACV for optimal growth prevention as well. Specifically for *Staphylococcus*, ACV did perform significantly better than yogurt ($p=0.04$) compared to the other species, which suggests that ACV is able to strongly inhibit the growth of *Staphylococcus* to a higher extent than for other species. Though the results for tea tree oil varied a bit more, it still showed promise in inhibiting growth in about half of the samples taken. Turmeric also had a few plates in which bacterial growth was prevented within a small circumference, but the results were not strong. Garlic had two outliers in which it showed inhibition, but for the most part there was no inhibition zone present. Both garlic and yogurt disks seemed to have additional tan-colored bacteria grown directly around them, indicating they contaminated the plate with outside bacteria and promoted their growth instead.

Conclusion

This study was successful in isolating and identifying the most prevalent bacteria located at the university's dairy. Apple cider vinegar showed a promising ability to be able to inhibit bacterial growth for the species seen in this experiment. Turmeric and tea tree oil showed a moderate ability, while yogurt and garlic were unsuccessful. Future studies can be conducted in order to determine if the concentration of each substance has an effect, as well as the efficacy of combining the substances together vs. keeping them separate. This data can be monumental for the medical and agriculture fields, as it can provide a natural, cost-friendly way to prevent bacterial infections. If they are utilized as treatments, it helps to avoid the overuse of antibiotics and avoid contributing to the problematic resistant bacterial strains that are emerging from this overuse. This is vital for human and animal health, as well as the status of the environment.

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