

## Introduction

*Borrelia burgdorferi* is the bacterium found in deer ticks which is responsible for Lyme disease. *Borrelia* enter the blood stream when a tick bites a human. Disease symptoms, including bull's eye rash, neurological complications, and arthritis, can take weeks, or even years to appear. According to the Center of Disease Control and Prevention, approximately 300,000 people are diagnosed with Lyme disease annually in the United States. *Borrelia* can take several forms: the spirochete, the cyst and the biofilm form. Biofilms are structured communities, encircled by a self-manufactured polymeric matrix to prevent *Borrelia* from adverse environmental conditions. Biofilms make the treatment of *Borrelia* extremely difficult as it can increase its resistance up to 1000 times, as opposed to individual spirochetes. Our research group has demonstrated that standard antibiotics have a very limited effect on *Borrelia* biofilm.

The purpose of this research project is to find a safe and effective antibacterial agent to combat *Borrelia* biofilm. The compounds chosen to investigate antibacterial effects on *Borrelia* biofilm were allicin and lactoferrin. Both allicin, a compound found in garlic, and lactoferrin, a protein commonly found in cow and human milk, were shown to have antimicrobial effects against a wide range of bacteria and have been used in the medical field to combat bacterial infections. Therefore, the effectiveness of allicin and lactoferrin were tested against *Borrelia* biofilm and compared to doxycycline, which is the standard antibiotic for Lyme disease treatment.

## Materials and Methods

Low passage isolates of B31 strain of *Borrelia burgdorferi* sensu stricto were cultured in Barbour-Stoner-Kelly H (BSK-H) complete medium supplemented with 6% rabbit serum. Serial dilutions of *Borrelia spirochetes* were grown for 6-days in a 48-well plate to initiate biofilm formation, followed by incubation with a tetrazolium dye, MTT (2 mg/ml in PBS) for 4 hours at 32° C. After incubation, the pellet was resuspended in either 150 µL of isopropanol and incubated on a rotatory shaker for 15 minutes. Absorbance of the supernatant was read at 570 nm using the Eon microplate spectrophotometer.

*Borrelia* biofilms were cultured as mentioned above and treated for three consecutive days (72 hours) with varying concentrations of antimicrobial agents such as allicin and lactoferrin and biofilm viability of *Borrelia* biofilms was determined using the MTT assay as described above.

As a positive control, 25 µg/ml of doxycycline (doxy), and as a negative control, the appropriate volume of PBS buffer, were used instead of antimicrobial agents. The two-sample paired t-test statistical analyses were performed using GraphPad Prism 6.00 for Mac (La Jolla, CA, USA).

## Results

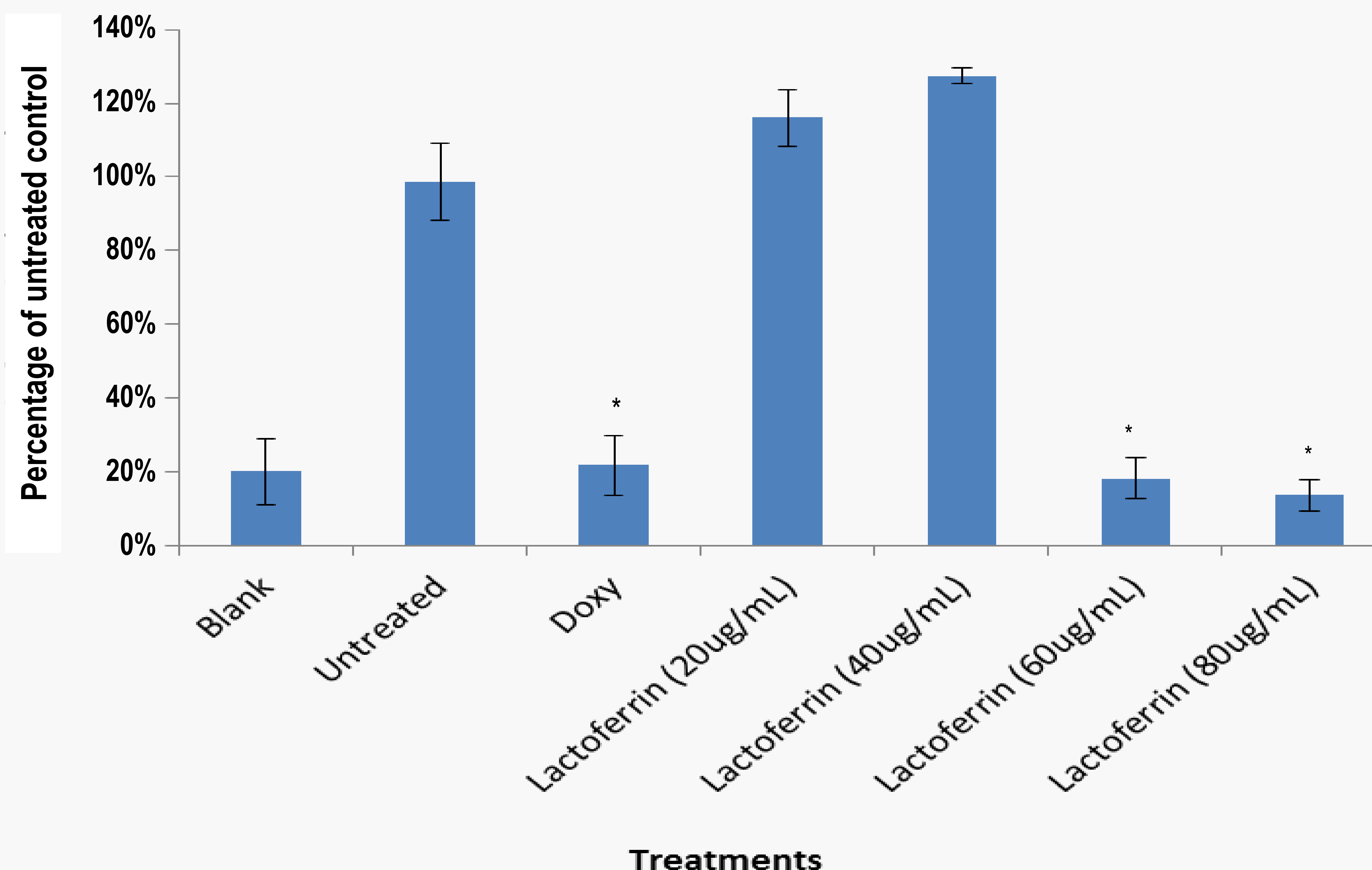


Fig 1. Quantitative analysis of *Borrelia burgdorferi* biofilm treated for 72 hours with Lactoferrin. MTT assay was performed on biofilm of *Borrelia* treated with different concentration of lactoferrin. Data was considered significant at p value<0.05. \* indicates p value≤ 0.05

## Conclusion

Data from this study showed that lactoferrin could have a significant effect on the viability of *Borrelia* biofilm, while allicin did not show any potential effect. The mechanism of the antimicrobial effect of lactoferrin has been previously suggested to work by absorbing and therefore sequestering iron, so bacteria cannot utilize it. In a recent publication in Nature, lactoferrin was demonstrated to significantly compromise the formation of biofilms of *Pseudomonas aeruginosa*. Lactoferrin chelated iron, disallowing *Pseudomonas* from taking up iron, causing "twitching, a specialized form of surface motility." This caused the bacteria "to wander across the surface instead of forming cell clusters and biofilms." This movement through *Pseudomonas* generations prevented organized biofilm structures to form so cells remained in a thin layer and were later proven to lose the antimicrobial resistance associated with organized biofilms. *Borrelia*, however, utilizes magnesium for biological processes instead of iron, which means that lactoferrin antibacterial effects on *Borrelia* biofilm must have a different mechanism which needs to be further investigated. Clinical studies with lactoferrin show no significant side effects and is a readily found protein in cow and human milk. Further research will be performed for my Honors Thesis where I plan to utilize additional tests to study the effect of lactoferrin on the different components of *Borrelia* biofilm, such as the protective mucopolysaccharide and extracellular DNA layers to better understand the mechanism of lactoferrin. Results from this research provided promising data for a safe, affordable, and highly effective approach to eliminate infections in Lyme disease patients.

## Discussion

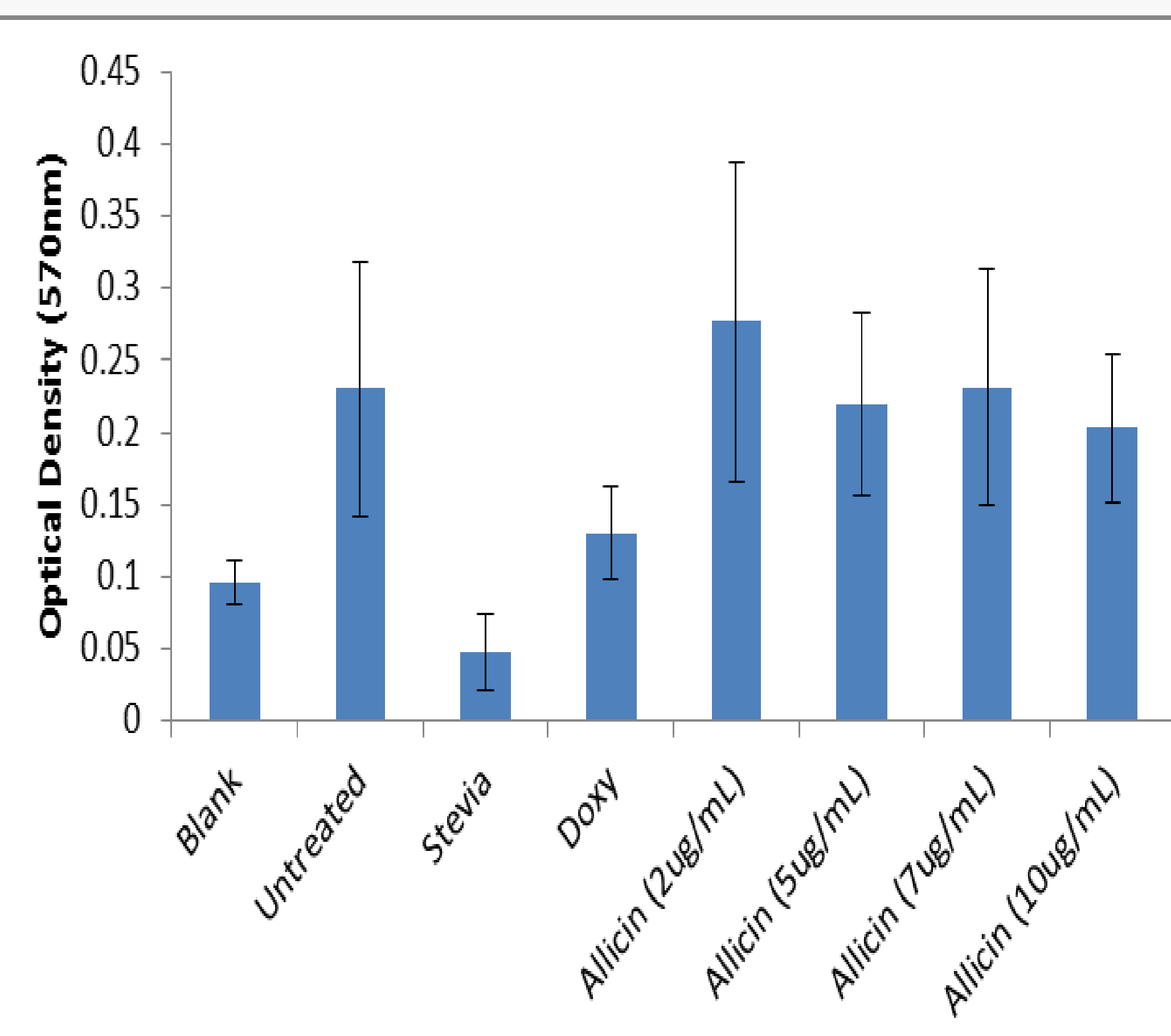


Fig 2. Quantitative analysis of *Borrelia burgdorferi* biofilm treated with different concentration of allicin using MTT viability assay. Negative control was a sample treated with vehicle (PBS buffer) and positive control had doxycycline (doxy) and Stevia, which was shown by previous research to have a significant effect on *Borrelia* biofilm.

The first compound tested was allicin but it was concluded after several MTT viability assays (Fig. 2) that allicin did not have a significant effect on *Borrelia* biofilm at any concentration studied. Then lactoferrin was chosen for the next round of tests. The experimental design included samples with a row of blank media, untreated *Borrelia* (negative control), doxycycline (positive control) and lactoferrin in various concentrations. It was important to test various concentrations to be able to determine what amount of the antimicrobial compound efficient in effectively eliminating the *Borrelia* biofilm. The concentrations tested were those used in literature, which varied from 20 ug/mL to 80ug/mL. Lactoferrin, at a concentration of 80 ug/mL, was the most effective and decreased the viability of *Borrelia* biofilm by 15% compared to the untreated sample. Comparing to positive control, lactoferrin was ~5% more effective than doxycycline, which is a compound commonly used in hospitals for treatment of Lyme disease.

Interestingly, lactoferrin at lower concentrations, such as 20ug/mL and 40ug/mL, increased *Borrelia* biofilm viability compared to the untreated control, however the effects were not significant.

## References

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