Abstract

Clostridioides difficile is a bacterial pathogen that causes antibiotic-associated intestinal disease. It is known for its role in antibiotic-associated diarrhea, colitis, and pseudomembranous enterocolitis. The infection can be severe, especially in immunocompromised patients, leading to a high mortality rate. Treatment options are limited, and preventive measures are crucial to reduce the incidence of C. difficile infections. The pathogenesis of C. difficile involves the production of toxins and the ability to form biofilms, which allows the bacteria to evade the host immune system and persist within the gut. Understanding the regulatory mechanisms that control C. difficile virulence is essential for developing effective therapeutic strategies.

Introduction

Clostridioides difficile is a gram-positive, spore-forming, obligate anaerobic bacterium. In humans, C. difficile colonizes the large intestines and is a leading cause of fatal nosocomial infections in the United States. Transmission is via the fecal-oral route, and disease is mediated by the release of two main exotoxins. These are toxin A, the enterotoxin, and toxin B, the cytotoxin. The first causes increased intestinal permeability and fluid displacement, while the second leads to colonic inflammation. Despite this knowledge, much of the basic biology of C. difficile is not well understood.

Cyclic diguanylate monophosphate (c-di-GMP) is a bacterial second-messenger molecule that is involved in the switch between planktonic motile and non-motile bacterial forms. An increase in the intracellular c-di-GMP concentration has been shown to induce exopolysaccharide synthesis and cellulase while inhibiting flagellar motility. C-di-GMP functions by binding to and influencing the activity of proteins and RNA riboswitches. A riboswitch is an RNA structure located in the 5' untranslated regions of some mRNAs that binds a specific metabolite; binding of the metabolite modulates the RNA structure in a way that alters expression of the downstream gene.

Phenotypic heterogeneity describes the presence of cells with distinct properties within an isogenic population. Phenotypic heterogeneity affects many aspects of the bacterial response to stimuli, and it is thought to increase bacterial fitness and the chances for survival of the population as a whole. Some survival strategies that are predicted to be affected by phenotypic heterogeneity include spore formation, biofilm formation, altered motility, and a differential response to antibiotics. As a whole, these strategies are evoked by environmental or bacterially produced signals. Depending on the stimulus, a change in the bacterial phenotype and response can occur.

In this study, we attempt to observe the effects of surface interaction on c-di-GMP binding, as well as identify whether C. difficile exhibits a population heterogeneity in the activation of the c-di-GMP riboswitch.

Methods

Streaked onto a plate

One colony was taken and placed in an overnight DilaGMP (Sigma-Aldrich) Concentration (µM) 0 1 2 3 DilaGMP binding to 1% of cell population in 1 hr.

Figure 1. Phase contrast and fluorescent images of the same frame.

Results

Average % fluorescence for cultures over 2 hrs

10 80

% of red fluorescent cells

% of red fluorescent cells

100

Conclusion and Future Study

In this experiment, we used a fluorescent reporter to compare the percentage of Clostridioides difficile cells exhibiting high c-di-GMP when grown in liquid media or on an agar plate. In the 2 hour experiment, the difference between the two cultures was minimal (<10% roughly). However, in the 24 hour experiment, there was a noticeable drop in the percentage of fluorescent cells at time 16 hours, suggesting a change in c-di-GMP levels. Since this was only performed once, more replicates are needed to test this point time. We also observed population heterogeneity: some cells showed higher red fluorescence than others, suggesting a difference in c-di-GMP levels. Mechanical errors, such as poor microscope resolution, made it difficult to record the total number of cells within a frame. For future studies, additional experiment trials will need to be performed to provide more accurate data, as well as an experiment to test why some cells exhibit more c-di-GMP riboswitch binding than others.

References