CLASSIFICATION OF CYSTIC FIBROSIS ISOLATES OF *P. AERUGINOSA* BASED ON TEMPERATURE SENSITIVITY

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Pseudomonas aeruginosa is a ubiquitous soil bacterium. Although harmless to most this bacterium has the ability to infect individuals with compromised immune systems such as those with a genetic condition called cystic fibrosis (CF). Chronic lung infection caused by *P. aeruginosa* is the major cause of mortality in patients with CF.

CF patients with lung disease have a robust immune response against a group of bacterial proteins known as heat shock proteins. The significance of this observation is not clear except for the association with increased inflammation. In this study, we evaluated whether such immune response in patients exerted a selective pressure causing the CF isolates to become temperature sensitive and how this happened. CF isolates obtained from the same patients from different geographic locations over a number of years were evaluated for growth characteristics at three temperatures: 37°C (body temperature), 42°C (heat shock response for *Escherichia coli*), and 45°C (heat shock temperature for *P. aeruginosa*). The CF isolates were first grown in 5 mL of tryptic soy broth (TSB) for 24 hours at 37°C. After this, 50 uL of the sample was transferred into another 5 mL tube of TSB, to be grown at the various temperatures for 24 hours. Using these samples, we obtained the optical density and plated them to grow overnight. From these plates, we calculated bacteria growth. This project's long-term goal was to discover the relationship between temperature sensitivity, genetic mutations and the pathogenesis of these mutations.

INTRODUCTION

According to a recent study by the Cystic Fibrosis (CF) Foundation, 30,000 people living in North America suffer from this genetic disorder. CF patients are predisposed to lung infection caused by various pathogens including *Pseudomonas aeruginosa*.[1] *P. aeruginosa* is an environmental bacterium which occurs in nature as a non-mucoid (non-slimy) form. When it colonizes in immunocompromised patients, such as those with CF, it converts from its non-mucoid form into a mucoid (slimy) form. This conversion is considered to be the leading cause of morbidity and morality in patients with CF.[2]

CF patients colonized with *P. aeruginosa* have a robust immune response against a group of bacterial proteins known as heat shock proteins, which as of right now, we only associate with increased inflammation.[3] In this study, we evaluated whether such immune response in patients exerted a selective pressure causing CF isolates to become more temperature sensitive and how this happened. Using CF isolates obtained from the same patients from different geographic locations over a number of years, we evaluated them for growth at three temperatures 37°C (body temperature), 42°C (heat shock response for *E.coli*), and 45°C (heat shock temperature for *P. aeruginosa*.)

The hypothesis of this study was that as the years go on, the patient from whom the organism was isolated will be more sensitive to the high temperatures due to mutations in the bacterium. When sequencing this isolate, we hoped to discover a link between temperature sensitivity and a gene that causes virulence in the CF isolate. The project's long term goal aimed to discover the relationship between temperature sensitivity, genetic mutation, and pathogenesis of these mutations.

METHODS

Using various *P. aeruginosa* laboratory strains (with VE19 as the control), we experimented with different techniques of testing temperature sensitivity.

Using CF isolates obtained from the same patients over a number of years, we evaluated them for growth characteristics at three temperatures 37°C (body temperature), 42°C (heat shock response for *E. coli*) and 45°C (heat shock temperature for *P. aeruginosa*.) These isolates were first grown in 5 mL of Tryptic Soy Broth (TSB) for 24 hours at 37°C.

After the overnight growth, 50 uL of this sample was transferred into another 5 mL tube of TSB and grown at the various temperatures for 24 hours. Using these samples, we obtained the optical density (OD). After obtaining this, we did serial dilutions. Using the OD, we made an accurate estimate of which dilutions to plate out on Tryptic Soy Agar as overgrowth of the bacteria made it difficult to calculate bacteria growth. After letting the bacteria grow overnight on the plate at 37°C, we calculated bacteria growth.

After analyzing the growth, we selected four isolates to be sequenced based on the first and last isolate of the patient. To sequence it, we grew the isolates overnight in TSB. From there, we followed the QIAGEN DNA purification kit protocol. We used four primers to sequence it.

RESULTS

We compared growth, at different temperatures, of the sequential isolates from the same patients over a period of 12 years as measured by optical density and plate counts. We noted the difference in the mucoid colony morphology of the CF21 at different temperatures, 37°C non-mucoid and 45°C mucoid and noted the different size of the colonies at various temperatures.

CONCLUSIONS

1) The isolates of *P. aeruginosa* from the same patients displayed a difference in temperature sensitivity during the course of chronic infection.
2) It appeared that most of the isolates that remained in the lungs of CF patients can not grow as well as the wild type bacteria at 45°C.
3) The temperature of 45°C is not lethal to the growth of bacteria. It only inhibited the growth of bacteria.
4) The same isolates displayed a different phenotype during the growth at different temperatures
5) The selected isolates mentioned earlier have been screened for mutations in 2 genes involved in the development of the mucoid phenotype. So far, a mutation has been found in an isolate from one patient at a late time that was absent early during the patient's disease.

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