Effects of pyocin production by *Pseudomonas aeruginosa*

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ABSTRACT

Cystic fibrosis (CF) is an inherited genetic disorder that results in an imbalance of chloride and sodium ions across apical cell membranes in the digestive system and the lungs. The result is thick mucus secretions that clog the lungs and leads to life threatening chronic infections by pathogens such as *Pseudomonas aeruginosa*. *P. aeruginosa* is quite prevalent as it is recovered from nearly 60% of all CF infections and contributes significantly towards morbidity and mortality. Antibiotic treatment rarely clears the bacterial populations from the lungs of CF patients due to increased antibiotic resistance and *P. aeruginosa*'s ability to produce biofilms. The difficulties encountered in treating patients with *P. aeruginosa* infections has increased interest in alternatives to traditional antibiotics and has inspired investigators to search for novel therapeutics. One such alternative includes antibacterial proteins called pyocins, which are produced by *P. aeruginosa* and could be adapted to target other *P. aeruginosa*. Pyocins are potent toxins with a narrow killing range in comparison to antibiotics. The aim of the proposed project is to evaluate the effects of pyocin production by *P. aeruginosa* in a *Drosophila melanogaster* infection model. *D. melanogaster* was infected individually and with mixed *P. aeruginosa* cultures consisting of pyocin producing *P. aeruginosa* and other CF isolates. The survival of *D. melanogaster* was monitored for 14 days. Mixed infections exhibited increased fly survival. Pyocin production was induced with the addition of Mitomycin C and assessed for by a bacteriocin production assay. The addition of Mitomycin C was found to increase pyocin production. The results demonstrate that pyocin production by *P. aeruginosa* may affect the survival of *D. melanogaster* when challenged with mixed *P. aeruginosa* infections.

INTRODUCTION

There are an estimated 30,000 cases of cystic fibrosis (CF) in the US today [1]. It is the most lethal genetic disease in white populations [2]. CF is a genetic disorder in which the gene that codes for CFTR, cystic fibrosis transmembrane conductance regulator, is dysfunctional [3]. CFTR is involved in sweat production, digestive fluids, and mucus. Therefore, CF patients often have excess mucus build up in the lungs that allows opportunistic infections to grow there. Once infected, CF patients frequently cannot clear infections due to the excess mucus [2]. One of the more prevalent and deadly infections is *Pseudomonas aeruginosa*. *P. aeruginosa* colonizes the lungs of the CF patient, develops resistance to many antibiotics used against them, and eventually leads to fatal lung deterioration [4]. Chest infections are typically the most serious symptoms for the CF patient. Therefore, because of the rise in antibiotic resistance in *P. aeruginosa* and many other bacteria, and the dangers of these infections in the CF patient, it is necessary to begin searching for novel treatments of CF lung infections [5]. There is a promising protein released by a certain strain of *P. aeruginosa* called pyocin. Pyocins have a narrow killing range and have been implicated in intra-species competition in nutrient limited environments [6] as well as mediating inter-species microbial diversity. As such, pyocins may be an effective alternative for mediating some of these long-standing CF lung infections.

METHODS

***Drosophila melanogaster* mixed infections.** Fly vials containing 6 mL of sucrose agar (5% sucrose and 2.2% agar) topped with a filter disc was used for *D. melanogaster* infections. Bacterial cultures were grown in Brain Heart Infusion (BHI) broth and normalized to $O.D._{600}$ = 3.0, centrifuged and the resulting cell pellet was resuspended in 150 µL of sterile 5% sucrose and added to the filter disks on top of the sucrose feeding vials. Control sucrose vials inoculated with 150 µL of 5% sucrose were used as negative controls in each experiment. *D. melanogaster* flies were then transferred to the sucrose feeding vials containing the bacterial suspensions, incubated at 25°C and fly mortality monitored daily for 14 days [6,7].

Growth inhibition plate assay: A modified assay of the pyocin typing assay developed by Fyfe *et al*. [8] was used. Briefly, isolates being tested for pyocin production were grown overnight in LB broth. Cultures to be induced for pyocin production were grown with Mitomycin C (1.5 ug/ml). After incubation the cultures were pelleted and the supernatants filter sterilized. A pyocin sensitive strain was grown overnight in LB, pelleted and the pellet used to inoculate a 5 ml culture of semi solid agar to an OD_{600} of 0.01. The inoculated semisolid agar was then poured onto a LB agar plate. Cell free supernatants of the test isolates were then spotted three times onto each plate. Diameters of the zones of clearing were measured.

Methodology behind mixed *P. aeruginosa* infections

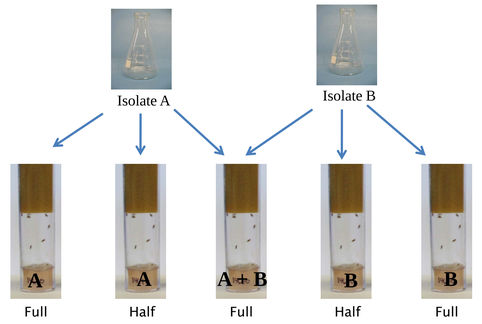

Figure 1

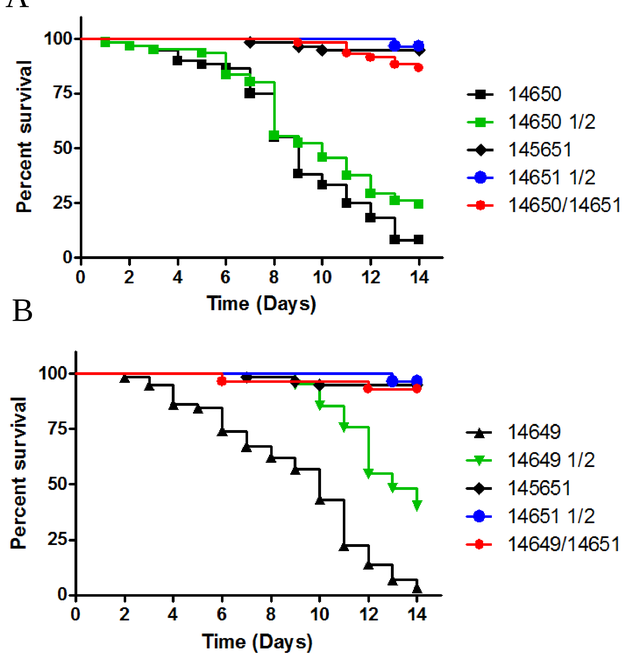

Figure 1: Mixed infection strategy. For mixed infections the total bacterial inoculum was held constant for each strain alone and also for the mixed infections. Mixed infections contained a 'half dose' (1 ml of an OD_{600} 3 bacterial culture per vial) of each strain, but in order to obtain a 'full dose' of total bacteria, twice the volume was used (2 ml of an OD_{600} 3 bacterial culture per vial). As controls we also tested the 'half dose' of each culture alone.

Figure 2: Mixed infections in the *D. melanogaster* fly feeding infection model. Five different infection conditions are shown: each isolate individually at a full dose (black lines), each isolate individually at a half dose (green and blue lines) and one mixed infection that consists of each bacterial isolate at a half dose (red line). CF isolates shown in (A) are 14650 and 14651 and (B) 14649 and 14651 were used to infect *D. melanogaster* in the fly feeding infection model. Fly mortality was measured daily. Data are representative of three or more replicate experiments, each performed in triplicate.

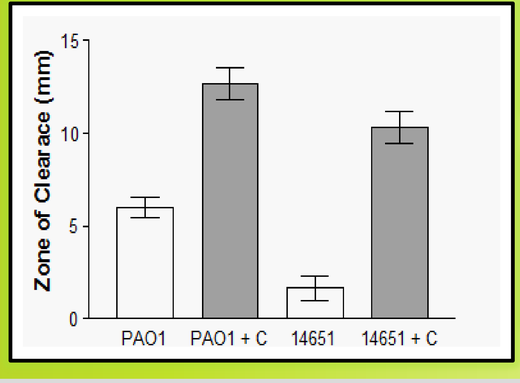

Figure 3: Inhibitory effects of PAO1 and CF isolate 14651 on a pyocin sensitive strain overlay. Cell free supernatants grown in LB or LB with Mitomycin C added were spotted on overlay plates of the pyocin sensitive CF isolate. Diameters of the zones of clearance were measured in mm. C=Mitomycin C, 1.5 ug/mL

CONCLUSIONS

Mixed infections of *P. aeruginosa* CF isolates exhibited a different infection profile compared to either isolate alone.

Mixed infections of 14649 and 14650 with 14651 showed decreased virulence in the *D. melanogaster* fly feeding model.

CF isolate 14651 was found to produce pyocins which may be contributing to the increased survival of the *D. melanogaster* flies during mixed infections.

FUTURE DIRECTIONS

Perform mixed infections using supernatants from 14651 either with or without the addition of Mitomycin C.

Determine susceptibility of 14649 and 14650 to pyocins produced by 14651 using growth inhibition assays.

Monitor survival of each isolate during *D. melanogaster* infection by measuring recoverable colony forming units during the course of mixed infections.

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