Characterization of Growth and Antibiotic Resistance in *Burkholderia Cepacia* Complex Organisms

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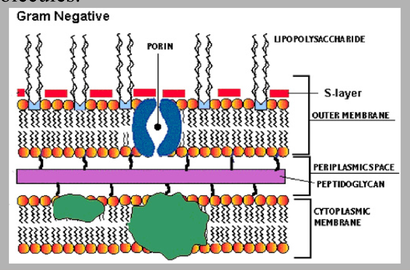
INTRODUCTION

The bacterial samples are from cystic fibrosis patients are a part of the *Burkholderia cepacia* Complex (Bcc). These opportunistic pathogens within the Bcc are phylogenetically related to *Pseudomonas aeruginosa*. Members of the Bcc are gram-negative bacteria. Gram-negative bacteria have a second outer membrane made up of lipopolysaccharides. This second outer membrane is generally impermeable to hydrophobic molecules. The purpose of this experiment is to identify the turbidimetric growth kinetics and the role the outer member plays in antibiotic resistance to hydrophobic molecules.



METHODS

Turbidimetric Growth Curves

The tests samples were placed in a shaking incubator for six hours at 180 rpm and 37 degrees Celsius, and every half hour a spectrometer was used to measure the optical density at 620 nm wavelength.

Disc Agar Diffusion Assay

Test samples were grown to 0.1 Optical Density to then be streaked on a petri dish with Mueller Hinton Agar. Eight different antibiotic discs were dispensed onto the agar. Discs diffused into agar in refrigerator at 3 degrees Celsius for the first hour, and then samples were placed in incubator for 24 hours at 37 degree Celsius.

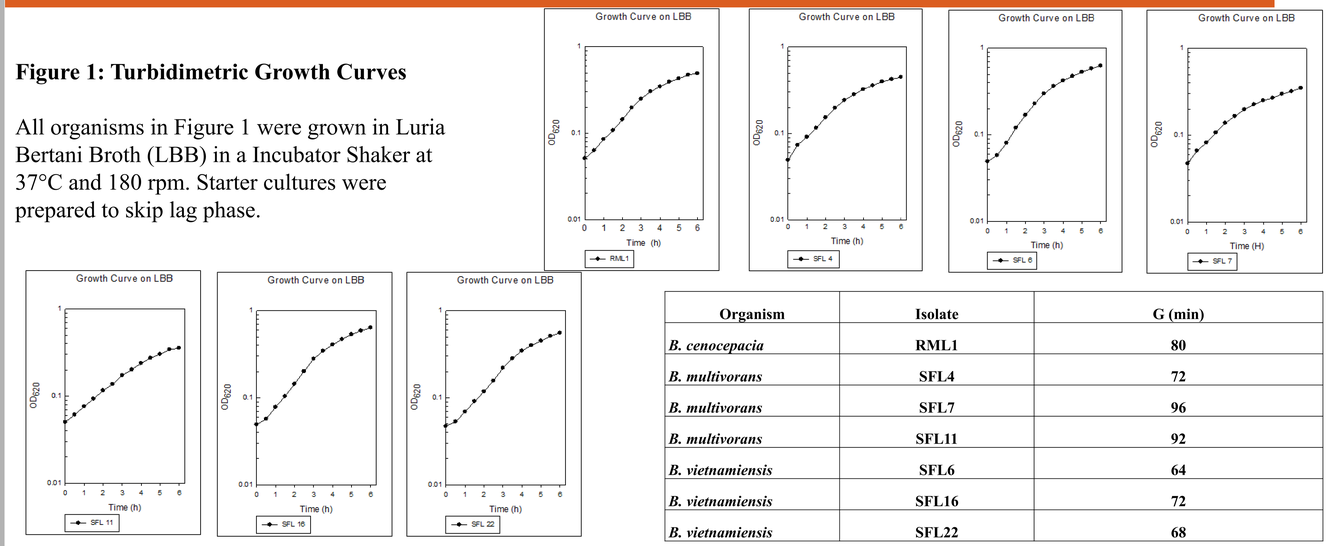
Incubator-Shaker

Spectrophotometer

RESULTS

Figure 1: Turbidimetric Growth Curves

All organisms in Figure 1 were grown in Luria Bertani Broth (LBB) in a Incubator Shaker at 37°C and 180 rpm. Starter cultures were prepared to skip lag phase.

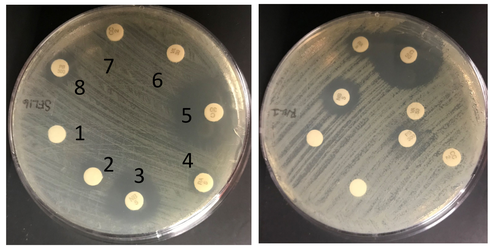


Organism	Isolate	G (min)
B. cenocepacia	RML1	80
B. multivorans	SFL4	72
B. multivorans	SFL7	96
B. multivorans	SFL11	92
B. vietnamiensis	SFL6	64
B. vietnamiensis	SFL16	72
B. vietnamiensis	SFL22	68

Table 1: Antibacterial Agent Susceptibility

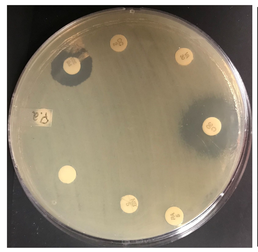
Organism	Strain	Ethanol	TSC	CHL	CLI	NOV	VAN	RIF	POL
P. aeruginosa	PA01	0±0	0.4±0.69	0±0	13.22± 4.58	0±0	0±0	0.95± 1.05	10.05± 0.69
P. aeruginosa	ATTC 27853	0±0	0±0	0±0	3.47± 3.72	0.06± 1.28	0±0	4.03± 1.63	10.37± 0.68
B. cenocepacia	RML1	0±0	3.08± 2.87	0±0	22.86± 4.51	8.46± 2.76	0±0	3.76± 1.26	3.21± 3.93
B. multivorans	SFL4	0±0	6.23± 0.90	0±0	15.45± 1.90	19.04± 2.67	0±0	3.43± 1.55	1.96± 1.96
B. multivorans	SFL11	0±0	2.70± 0.39	0±0	8.03± 1.28	11.23± 2.12	0±0	0.86± 1.49	0±0
B. vietnamiensis	SFL6	0±0	3.87± 0.70	0±0	15.89± 2.92	8.53± 4.04	0±0	4.81± 0.64	1.11± 0.96
B. vietnamiensis	SFL16	0±0	3.77± 1.00	0±0	16.44± 3.35	10.39± 2.86	0±0	3.22± 0.68	5.1± 3.58
B. vietnamiensis	SFL22	0±0	5.43± 2.53	0±0	17.81± 4.80	13.64± 3.28	0±0	5.38± 2.46	5.36± 4.65

Figure 2: Disc Agar Diffusion Bioassay

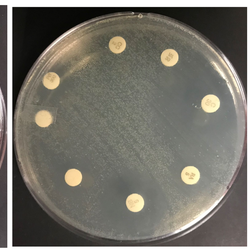


B. vietnamiensis SFL16

B. Cenocepacia RML1



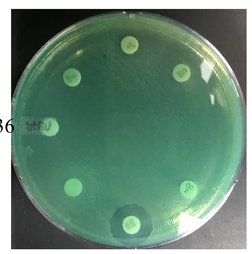
B. multivorans SFL11



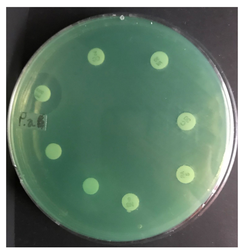
B. vietnamiensis SFL22

Table 1. ETOH is 95% ethanol, TSC is triclosan (0.2 µg), CHL is chloramphenicol (30 µg), CLI is clindamycin (1.0 µg), NOV is novobiocin (5.0 µg), VAN is vancomycin (30 µg), RIF is rifampin (5.0 µg), and POL is polymyxin B (≈36 µg)

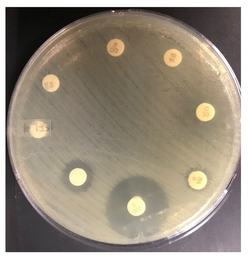
Figure 2. 1- ethanal, 2- triclosan, 3- novobiocin, 4- rifampin, 5- clindamycin, 6- vancomycin, 7- chloramphenicol, 8- polymyxin B.



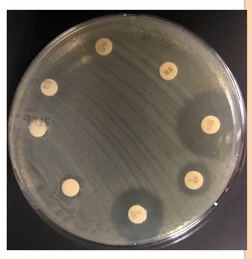
P. aeruginosa ATCC 27853



P. aeruginosa PAO1



B. multivorans SFL4



B. vietnamiensis SFL6

RESULTS SUMMARY

Turbidimetric Growth Curves

- All isolates exhibited typical sigmoid growth kinetics under batch cultural kinetics as expected.
- All examined isolates grew at relatively similar rates (range of 68-96 min) and yielded similar amounts of total biomass.
- SFL 6 resulted in the most biomass during the 6-hour trial.

Disc Agar Diffusion Assay

- Both *Pseudomonas aeruginosa* control strains were resistant to triclosan and susceptible to polymyxin B to about the same degree.
- All isolates were slightly susceptible to triclosan at approximately the same levels.
- All of the Bcc isolates were slightly to moderately susceptibility to triclosan.
- The Bcc test isolates were all resistant to chloramphenicol, moderately to very susceptible to clindamycin, moderately to very susceptible to novobiocin, resistant to vancomycin, and resistant to susceptible to rifampin.

CONCLUSION

Turbidimetric Growth Curves

- The typical sigmoid growth curves indicate that all the Bcc isolates were pure.
- Division was by transverse binary fission in all cases.
- All isolates grew well albeit slowly on Luria Bertani Broth at 37°C under batch conditions.

Disc Agar Diffusion Assay

- All of the Bcc organisms exhibited similar patterns of resistance to various hydrophobic antibacterial agents in the antibiogram.
- The outer membranes of Bcc organisms examined are clearly permeable to certain hydrophobic molecules, therefore outer membrane exclusion does confer resistance to all hydrophobic molecules.

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