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Title: Effect of short-chain fatty acids and pH on aerobic and anaerobic growth of *Pseudomonas aeruginosa*

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Body: Background: *Pseudomonas aeruginosa* (PA) is one of the most common pathogens in patients with cystic fibrosis (CF) lung disease. PA and other anaerobic bacteria produce short-chain fatty acids (SCFAs) as byproducts of anaerobic fermentation. SCFAs are more volatile at low pH values, and reports have shown that CF patient sputum is acidic. Recently, we detected SCFAs in the low millimolar range in the sputum of CF patients. Aim: To determine if SCFAs affect bacterial growth at different pH levels. Methods: The laboratory strain PAO1 and a clinical isolate PA508 were used for experiments with and without the addition of SCFAs (acetate, propionate, butyrate) at a range of concentrations between 3.125-100 mM and pH values from 5.5-7.0 in tryptic soy broth. Optical density at 600 nm was measured every 30 minutes for a 6 hour period, as well as viable colony counts after the period of incubation. Anaerobic/microaerobic conditions were generated by sealing microplates with optically-clear PCR film. Results: Acetate showed a significant inhibition in growth above 50 mM (>77%, $p<0.0001$) that was dependent on pH. Conversely there was a significant increase in growth below 12.5 mM acetate (>20%, $p<0.05$). Propionate had the highest inhibition, followed by butyrate and acetate. Anaerobic conditions resulted in lower growth overall and showed similar trends in growth inhibition. Results were consistent for both PA strains tested. Conclusion: SCFAs increase the growth of laboratory and clinical strains of PA at concentrations found in CF patient sputum, but inhibit growth at high concentrations. SCFAs produced by anaerobic bacteria may provide an indication of increased virulence of PA.