Flow cytometric monitoring of a three-species mixed bacterial community relevant for Cystic Fibrosis

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Abstract

The lung of Cystic Fibrosis (CF) patients is chronically infected by a large number of different pathogens coexisting in mixed bacterial communities. Chronic infection cause persistent inflammatory response leading to lung destruction and loss of lung function. Predominant species in those communities are upon others Pseudomonas aeruginosa, Burkholderia cepacia and Staphylococcus aureus. Interspecies interactions, synergistic as well as antagonistic, contribute to the complexity and the severity of such a chronic infection and therefore

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have to be considered in antibiotic therapy. Only few reports are available about the influence of microbial interactions on growth dynamics of single species in CF-relevant communities. To address this, a defined mixed bacterial community, consisting of P. aeruginosa, B. cepacia and S. aureus grown in shake flask cultures on complex medium was monitored by flow cytometry using a newly developed 3-color staining assay. Briefly, to discriminate bacteria from electronic background noise, the samples were stained with DAPI against DNA. For species-specific detection, a fluorescently labelled antibody specific for B. cepacia and fluorescently conjugated wheat germ agglutinin specific for gram positive bacteria were applied. The new assay was tested successfully in pure and mixed cultures and enabled clear discrimination between P. aeruginosa, S. aureus and B. cepacia. From time series experiments growth characteristics on the single cell level for each species were obtained in pure and mixed cultures. Growth of S. aureus was inhibited significantly in mixed culture, whereas growth of P. aeruginosa and B. cepacia were comparable in pure and mixed culture. These results suggest inhibition of S. aureus through P. aeruginosa and B. cepacia. Furthermore cell lysis of S. aureus was observed in mixed culture, which indicates production of staphylolytic extracellular proteins by P. aeruginosa and B. cepacia. Finally, these results are discussed in the context of other recent proteomic data from our group, which correlate well with the described findings.

Keywords: mixed communities, flow cytometry, proteomics, Cystic Fibrosis, growth dynamics, interactions