Using stable isotope tracers to elucidate the *in situ* metabolic activity of microbial populations in the cystic fibrosis lung

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Background

Cystic fibrosis is a genetic disorder that causes the accumulation of mucus in a variety of organs. The genetic defect allows for the colonization and chronic infection of the pulmonary system by opportunistic pathogens and ranks as the most common lethal genetic disorder in Caucasian populations. The formation of complex microbial communities and their physiological heterogeneity within the cystic fibrosis lung are suspected to contribute to rapid disease progression and antibiotic resistance. However, because of the inherent challenges of working *in situ* with human patients, very few studies of the *in situ* metabolic programs of infectious agents exist and the development of effective antimicrobial therapies is often hindered by our limited knowledge of the physiological state of microbial populations within the human host. It is currently unknown how even basic metabolic indicators, such as microbial growth rates, may correlate with disease progression.

Discussion

Here, we present a novel approach based on techniques developed in the (bio)geochemical sciences to tackle this knowledge gap. Using ^{2}H and ^{15}N isotope tracers, combined with GC-IRMS and nanoSIMS analytical techniques, we characterize the growth rate, community structure and metabolic state of microbes within the lungs of cystic fibrosis patients. Contrary to the traditional model of a rapidly multiplying infectious population, our results from compound specific lipid analysis of biosynthetic ^{2}H incorporation suggest microbial activity consistent with slow growth (a physiological adaptation known to confer antibiotic resistance in the laboratory). At the same time, analyses of spatial variation in microbial activity using fluorescent *in situ* hybridization and nanoSIMS highlight metabolic heterogeneity within the microbial community. This approach provides a first glimpse at the *in situ* biosynthetic activity of pathogens involved in chronic infection of the cystic fibrosis airways and contributes to building a basis for physiologically informed laboratory experiments.

Highly dynamic cellular-level response of symbiotic coral to sudden increase in environmental nitrogen

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Metabolic interactions with endosymbiotic photosynthetic dinoflagellates *Symbiodinium* sp. are fundamental to reef-building corals (Scleractinia) thriving in nutrient-poor tropical seas. Yet detailed understanding at the single cell-level of nutrient assimilation, translocation, and utilization within this fundamental symbiosis is lacking. Using pulse-chase ^{15}N-labeling and quantitative ion-microprobe isotopic imaging (NanoSIMS) we visualized these dynamic processes in tissues of the symbiotic coral *Pocillopora damicornis* at the sub-cellular level. Assimilation of ammonium, nitrate, and aspartic acid resulted in rapid incorporation of nitrogen into uric acid crystals (after ~45 minutes), forming temporary N-storage sites within the dinoflagellate endosymbionts. Subsequent intracellular remobilization of this metabolite was accompanied by translocation of nitrogenous compounds to the coral host, starting at ~6 hours. Within the coral tissue, nitrogen is utilized in specific cellular compartments in all four epithelia, including mucus chambers, Golgi bodies, and vesicles in calicoblastic cells. Our study shows how nitrogen-limited symbiotic corals take advantage of sudden changes in nitrogen availability and opens new perspectives for functional studies of nutrient storage and remobilization in microbial symbioses in the changing reef environment.

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