DEBORAH MARY MOORE\$, HONGWEI YU, T. RYAN WITHERS, and WENDY TRZYNA, Department of Biological Sciences, Marshall University, Huntington, WV, 25755, Biomedical Sciences, Marshall University, Huntington, WV, 25755. Interaction and intracellular localization of Pseudomonas aeruginosa strains with Acanthamoeba castellanii.

Acanthamoeba spp. are single celled eukaryotic microbes found within a wide variety of environmental niches. Acanthamoeba have a dual lifecycle consisting of an active feeding trophozoite stage, and a dormant cyst stage that enhances their survival when environmental stress is encountered. These protozoa typically feed upon bacteria found within their environment with some bacterial species existing as endosymbionts, while other highly virulent bacterial strains may kill the amoebae. It is also possible that pathogenic bacterial strains that inhabit Acanthamoeba, in order to facilitate their survival, may be capable of undergoing changes such as increased virulence which could enhance invasiveness of the bacteria within their eukaryotic hosts. Pseudomonas aeruginosa, a gram negative environmental bacterium, known to cause disease was labeled with green fluorescent protein (GFP) as a marker for tagging the bacterial strains of interest that may co-exist within Acanthamoeba. These bacterium were then targeted for their intracellular location within amoeba via fluorescent microscopic analysis. Acanthamoeba cells that had been placed in nonnutrient medium for selected durations were co-cultured with a highly virulent strain PAO1V, and a non-virulent mutant strain PAO1V Δ aroa. for different time intervals. Preliminary results show localization of GFP-labeled bacterial cells within Acanthamoeba with clusters of bacterial cells immediately surrounding amoeba cell walls, and more concentrated in certain vacuoles. Determination of the exact intracellular location of these bacteria within Acanthamoeba was made. Further studies into whether bacterial cells are present within amoeba during encystment may also provide a better understanding of this interaction taking place.