Complex interactions between pathogenic bacteria, the microbiota, and the host can modify pathogen physiology and behavior. We describe two different experimental approaches to study microbe-microbe interactions in in vitro systems containing surface-associated microbial populations. One method is the application of RNA sequencing (RNA-seq) to determine the transcriptional changes in pathogenic bacteria in response to microbial interspecies interactions. The other method combines flow cell devices for bacterial cultivation and growth with high-resolution bioimaging to analyze the microscale structural organization of interacting microbial populations within mixed-species biofilms.