

reservoirs. To disseminate within and between these hosts, spirochetes must migrate through complex, polymeric environments such as the basement membrane of the tick midgut and the dermis of the mammal. To date, most research on the motility of *B. burgdorferi* has been done in media that do not resemble the tissue milieu that *B. burgdorferi* encounter *in vivo*. Here we show that the motility of *Borrelia* in gelatin matrices *in vitro* resembles the pathogen's movements in the chronically infected mouse dermis imaged by intra-vital microscopy. More specifically, *B. burgdorferi* motility in mouse dermis and gelatin is heterogeneous, with the bacteria transitioning between at least three different motility states that depend on transient adhesions to the matrix. We also show that *B. burgdorferi* is able to penetrate matrices with pore sizes much smaller than the diameter of the bacterium. We find a complex relationship between the swimming behavior of *B. burgdorferi* and the rheological properties of the gelatin, which cannot be accounted for by recent theoretical predictions for microorganism swimming in gels. Our results also emphasize the importance of considering borrelial adhesion as a dynamic rather than a static process.

756-Pos Board B542

Visualization of Vortical Flow Patterns in a Thin Fluidic Biofilm Surrounding a *Bacillus Subtilis* Bacterial Colony

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The collective behavior of bacteria plays an important role in the biofilm development. It is essential to investigate the dynamics of bacterial colonization and the interactions between the bacteria and the physical parameters of the substrate and fluidic biosurfactants as well. Recently, studies on prokaryotic cell biology have been facilitated with the advancement of quantitative microscopic imaging techniques. In this study, the fluidic properties of the biofilms formed in *Escherichia coli* (*E.coli*) and *Bacillus subtilis* (*B.subtilis*) colonies were compared by visualizing 200-nm fluorescent beads which were initially embedded in the agar plate and distributed spontaneously on the upper surface of the growing colonies. We focus on finding the effect of the substrate stiffness on their swarming motility and on the hydrodynamics of the fluidic biofilm by long-term live cell imaging of *B.subtilis* cells grown on an agar gel substrate. The viable cell number and motility of the bacteria in the colony were measured by phase contrast imaging and multiple particle tracking techniques. It was found that the bacteria in the biofilm have a property to switch between the growth and swarming phase. Vortical flow patterns near the edge of the colony were observed clearly by tracking of the beads moving in the biofilm of the *B.subtilis* colony, whereas there was no movement of the beads in the *E.coli* colony. Further analysis with a cross-correlation method revealed the detailed velocity vector field of the thin biofilm surrounding the *B.subtilis* cells. The present study should be a first step to find the effect of the fluidic biofilm on the growth and the swarming dynamics of bacteria, and to uncover a novel physical factor that regulates bacterial cell physiology.

757-Pos Board B543

The Growth of Bacterial Colonies

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On hard solid media (agar gel), there is often insufficient surface hydration for bacteria to swim or swarm. Instead, bacteria in the colony are close packed and spreading occurs through elastic repulsion: individual bacteria push each other out of the way through the force of their growth. It has long been known that, under these conditions, colonies grow linearly in dimension rather than exponentially as they do in liquid media of the same composition. We explore

this phenomena using a combination of confocal microscopy experiments to probe the full three-dimensional structure of the colony during growth, and numerical simulations of the cellular dynamics. Results indicate that nutrient limitation is the primary factor leading to linear growth. The complete evolution of the colony shape with time can be explained by modeling the cells as a granular fluid with active expansion due to growth.

758-Pos Board B544

Modeling the Dynamics of *Escherichia Coli* Populations through Spectroscopy and Particle Tracking

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Developing predictive models of microbial communities is of current interest in microbial ecology (1). *Escherichia coli* cultures constitute good model system to study in this context. *E. coli* cultures have been investigated in terms of metabolism, biochemical reactions, and genetics. However, as opposed to biochemical dynamics, spatial (physical) dynamics has been less studied, especially with regard to bacteria-predator relationships. We construct a statistical analysis method for analysis of *E. coli* dynamics grown in the presence of a predator bacterium, *Bdellovibrio bacteriovorus*. Measurements are performed by optical microscopy and tracking, as well as by NMR spectroscopy. Variation over time with respect to predator bacteria or chemical agents of cell death are monitored by ³¹P NMR spectra, population density, and tracking parameters of *E. coli*. Phosphorus NMR is used to determine the distribution of phosphates within cells. Spectra are compared between cells during early, middle, and late logistic growth, cells killed by chemical agents, e.g. ethanol or sodium hypochlorite, and cells grown in the presence of the predator. Population density is measured by light scattering at 600 nm under the various conditions listed above for NMR. Results could help construct a dynamic model of bacterial populations. With such a model, ecological processes resulting in various population fluctuations might be described at a fundamental physical level and predicted more accurately. [1] McMahon et al. *Current Opinion in Biotechnology* 2007, 18, 287.

759-Pos Board B545

Imaging Bacterial Colonization of the Zebrafish Gut with Selective Plane Illumination

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Microbes are a major component of living animals, outnumbering the human cells in humans, for example, by an order of magnitude. The mechanisms by which resident microbial communities assemble, however, are very poorly understood, in large part due to the difficulty of imaging microbes inside animals and to the scarcity of tractable model systems. Selective plane illumination microscopy (SPIM), also known as light sheet fluorescence microscopy, is a recently developed imaging technique that is able to acquire high-speed three-dimensional images over long periods of time with high resolution, a wide field of view, and low levels of phototoxicity. Zebrafish (*Danio rerio*) are well-studied model vertebrates that are transparent during larval life when their intestines are first colonized by bacteria. We can rear germ-free zebrafish that can then be inoculated with particular bacterial species expressing fluorescent proteins. Using a home-built SPIM microscope, we have begun to investigate the dynamics of bacterial colonization of the larval zebrafish intestine. We provide here the first demonstration that SPIM has the resolution and speed necessary to image individual bacteria as well as larger colonies over periods of several hours. Imaging colonies allows quantification of changes in the spatial distribution of bacterial populations, while imaging individuals makes it possible to explore the motility of these organisms in more natural settings than conventional culture-based assays allow.