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Title:	Exploring the impact of three-dimensional confinement on bacterial growth dynamics
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Abstract:	For centuries, bacteria have been studied in homogeneous liquid cultures or on 2D sur- faces. However, natural habitats of bacteria, such as mucus or soil, are granular, porous, and three-dimensional. Furthermore, the mechanical properties of these natural environ- ments are highly variable and bacteria can sense and respond to these changes in their microenvironment. To understand the changes in the growth dynamics of bacteria in condi- tions similar to their natural habitat, we utilize an already known 3D system of a jam-packed polyelectrolyte microgel. This is a porous, granular, 3D system that is viscoelastic in nature and mimics the physical properties of gut mucus. We used bacterial strains isolated from the gut of red-flour beetle to show that 3D confinement has an effect on overall bacterial growth. We also report that the single-cell morphology of the bacteria influences the shape of the bacterial colony, which in turn determines the availability of nutrients for the bacte- ria, thereby controlling the overall population growth. Polyelectrolyte microgels are capable of sequestering charged molecules such as antibiotic kanamycin and other ions. To conduct more studies in 3D, it is very necessary to have a versatile biomaterial. To overcome this barrier, we develop a charge-neutral jammed micro- gel system with a simple one-step synthesis protocol using a common laboratory reagent, agarose. This 3D system can be prepared in any growth medium such as LB, YPD, or RPMI and thus can be used to culture bacteria and yeast. Because of the charge-neutral nature of our microgel, charged selection agents such as kanaymcin can be used without compromising its efficiency. Our charge-neutral agarose microgel can be used in the future to study host-pathogen interactions in 3D granular environments.
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