**Novel Results: Microfluidics that replicate the ground beneath root systems shows the evolution of microbial biofilms**

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<th>Objective</th>
<th>• Develop a microfluidic system to study the flow-induced spatial evolution of bacterial biofilms underground.</th>
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| New science | • A complex, two-dimensional, microfluidic platform was designed that retained the pore-scale physical structure of porous media while providing a fully defined, tractable model for characterizing pore space hydrodynamics.  
• Both wild type *Pantoea* sp. YR343 and a biofilm-defective mutant (ΔUDP) were observed in the device and rheotaxis (directed bacterial movement across a velocity gradient) has the biggest influence on spatial distribution of bacteria.  
• There was no significant growth differences between the two strains, but both strains exhibited a different phenotype, which was dependent upon flow within the pore space.  
• These phenotypes subsequently influenced the overall spatial distribution of cells across the porous media as colonies grew and altered the fluid dynamics of their microenvironment. |
| Impact | • We replicated both the two-dimensional and packing distributions of natural sand in a 2D microfluidic platform, which enabled quick data capture to study the dynamics of bacteria-grain interactions.  
• This method can be used to further characterize the spatial evolution of bacterial biofilm in a porous network. |


A microfluidic platform replicates the natural shape and layout of sand grains. (A) The device, filled with fluorescein to highlight its features, has a bifurcating inlet and outlet to uniformly distribute bacterial cells across the design (C) The porous network design (approximately 6,000 x 6,000 x 10 μm) consists of heterogeneous pore spaces that have (B) constriction points smaller than the size of a bacteria cell and (D) larger, highly connected pore spaces. (E) Velocities within the pore space were simulated using COMSOL Multiphysics, which illuminated multiple preferential flow paths across the system. (F,G) The pore space velocities were experimentally verified using particle image velocimetry and velocity magnitudes (F, regions 1, 2, 3, decreasing in magnitude) corresponded to bead speeds within the same pore (G, regions 1, 2, and 3) (scale bars = 100 μm, velocity units = m/s).