Role of *Bacillus Subtilis*, A Plant Growth-Promoting Rhizobacteria, in Improving Soil Hydro-Physical Properties

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**Introduction**
- *Bacillus subtilis* is well-known for certain PGPB (Plant Growth Promoting Rhizobacteria) qualities, but the exact mechanism(s) in influencing evaporation and water retention is not well understood.
- As a stress-tolerant bacteria, *Bacillus subtilis* can produce visco-elastic biofilm to cope with the fluctuating soil water conditions. This, in turn, can affect the hydraulic and interfacial properties of soil.
- Identifying key missing links between the important physicochemical traits of the early-stage biofilm and EPS (Extra-cellular Polymeric Substances) of *Bacillus subtilis* and soil physical and hydraulic properties is the motivation of this work.
- Research outcomes may contribute to developing biological strategies to increase soil water retention and crop production in arid regions.

**Objectives**
- To investigate water percolation and evaporation from sand inoculated with EPS-producing *B. subtilis* strain FB17 (wild-type) and its EPS-knockout mutant (eps);
- To elucidate the mechanisms by which the physicochemical properties of EPS affect water-related physical and hydrological properties of sand.

**Materials and Methods**
- **Treatments**: Wild-type (*B. subtilis*, FB17) and eps (EPS-knockout mutant).
- **Column experiments**:
  - Column dimension: Φ4.37x0.66 cm;
  - Sand size: 0.297-0.42 mm;
  - Bacterial conc: 7x10^7 CFU/g;
  - Initial water content (w/w): 5%;
  - Infiltration amount: 20 ml dye water (x-field capacity water content)
- **Complementary Measurements**:
  - Water retention curve (WRC);
  - Pellicle assay;
  - Scanning electron microscope (SEM) imaging;
  - Contact angle, viscosity & surface tension.

**Possible Mechanisms**
- Increased viscosity or viscous dissipation length (L_v) lowered evaporative loss by reducing the critical drying front depth (L_c) (Lehmann, et al., 2008).
- Higher contact angle and lower surface tension of the strains disrupted the water film connectivity to reduce evaporation loss according to the Young-Laplace equation:
  \[ \frac{L_v}{L_c} = \frac{\sigma_{visc}}{\sigma_{water}} \]
- The dampening effect of viscosity maintained the connectedness at the drier end (occurrence of Rayleigh instability in the presence of EPS at low Reynolds number).
- The interplay between surface tension and visco-elasticity led to complex changes in water retention and flow in treated sand.

**Results – Percolation & Water Distribution**
- Inoculation with both the wild-type and eps mutant reduced percolation rate.
- Bacteria-treated columns showed heterogeneous water distribution pattern whereas water distribution in the control was homogenous.
- The results suggest hydraulic decoupling.

**Contact Angle and Surface Tension**
- Both strains increased contact angle of treated sand and reduced surface tension of LB solution.
- Water droplet persistency showed reduced wetting or hydraulic stabilization by the wild-type.

**Viscosity & EPS Composition**
- **G > 0** means more elastic;
- **G =** Total viscosity;
- Wild-type EPS is more visco-elastic than the mutant;
- Carbohydrate compositions are similar.

**Conclusions**
- Both bacterial strains increased water retention of a fine sand during both upward (percolation) and downward (evaporation) flow of water.
- Interrupted capillarity, decreased wetting and hydraulic decoupling are likely the causative mechanisms for these observations.
- Structural development is not an essential mechanism in EPS-driven water retention at the early-stages of biofilm formation.
- The results clearly demonstrate the effects of surface tension, viscosity and their interplay on water behavior in bacteria-treated media.
- The similar effects observed for both the wild-type and mutant strains of *B. subtilis* highlight the complexity and our limited understanding on the mechanisms through which PGPBs mediate changes in soil water status.

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