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

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Introduction

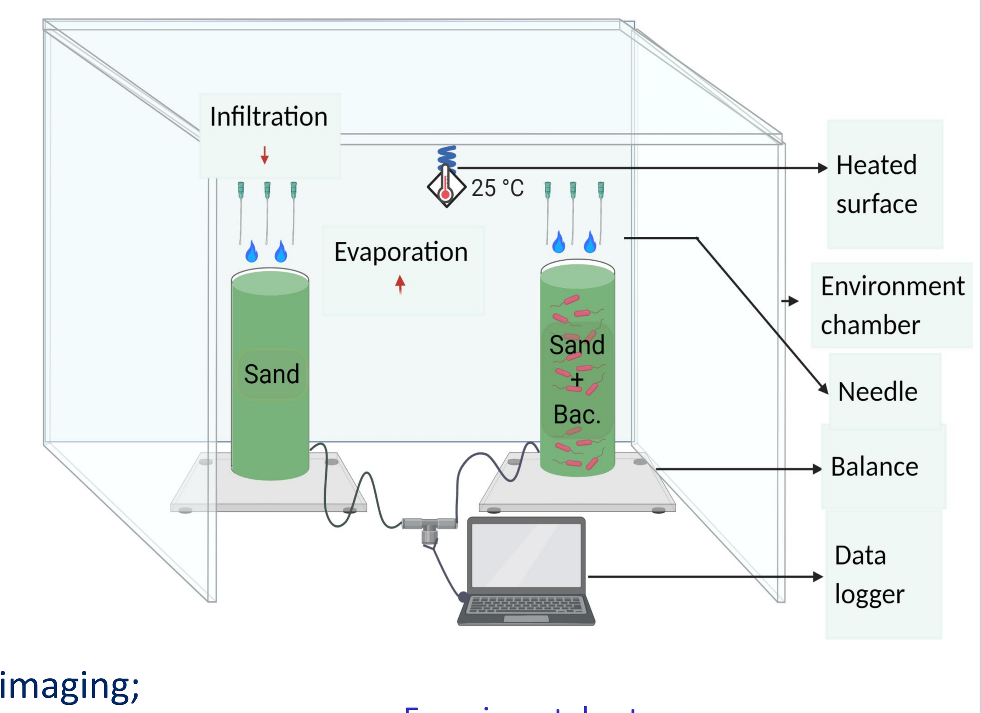
- *Bacillus subtilis* is well-known for certain PGPR (Plant Growth Promoting Rhizobacteria) qualities, but the exact mechanism(s) in influencing evaporation and water retention is not well understood;
- As a stress-tolerant bacteria, *Bacillus subtilis* can produce visco-elastic biofilm to cope with 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: ø4.37x9.66 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 (~ field capacity water content).
- **Complementary Measurements:**
  - Water retention curve (WRC);
  - Pellicle assay;
  - Scanning electron microscope (SEM) imaging;
  - Contact angle, viscosity & surface tension.

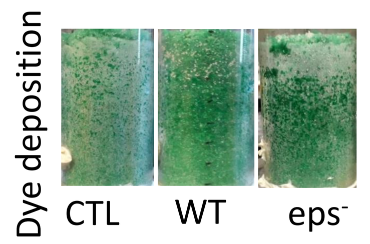


Experimental set-up.

Results – Percolation & Water Distribution

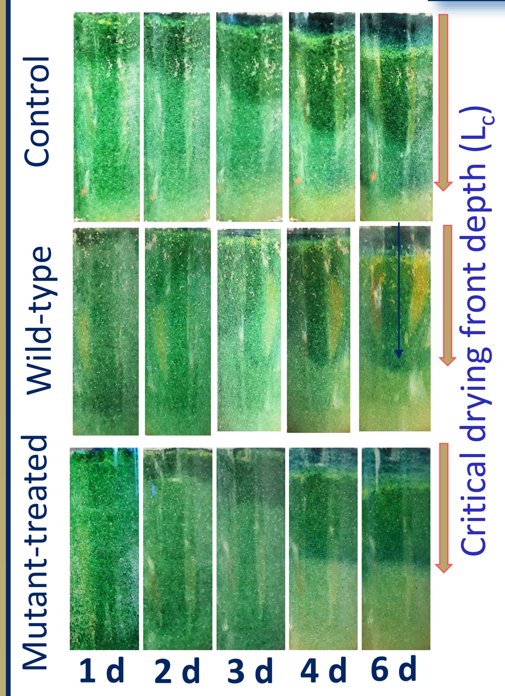
	CTL	WT	eps⁻	CTL	WT	eps⁻	CTL	WT	eps⁻
Rate (cm/ sec)	0.17	0.14	0.13	0.13	0.11	0.09	0.1	0.09	0.08

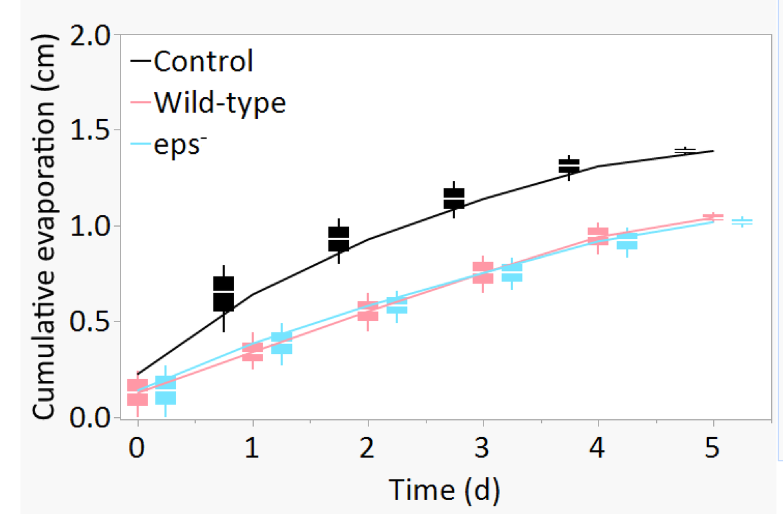
CTL= Control
WT= Wild-type
eps⁻= Mutant

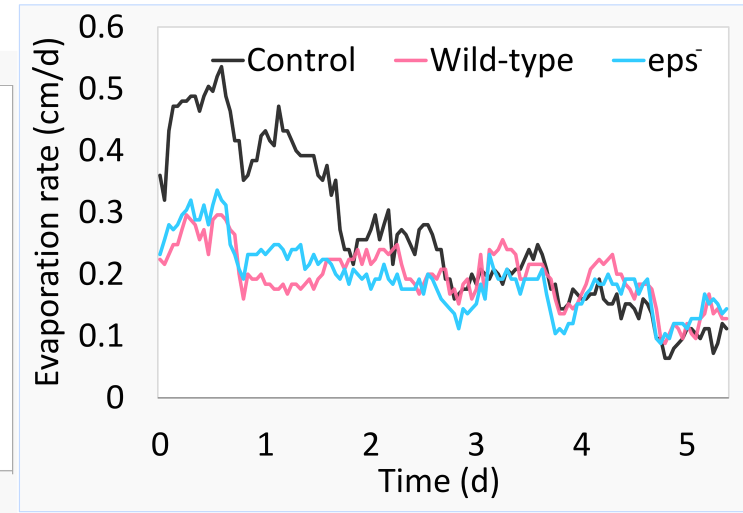


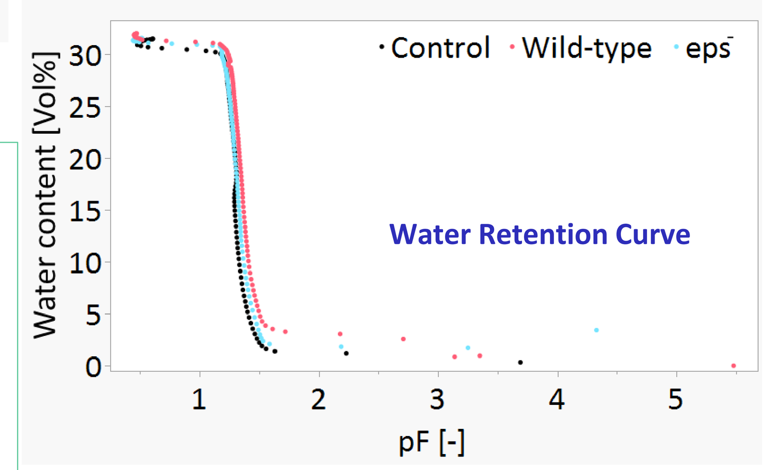
- Inoculation with both the wild-type and eps⁻ mutant reduced percolation rate;
- Bacteria-treated columns showed heterogeneous water distribution pattern whereas water distribution in the control was homogeneous;
- The results suggest hydraulic decoupling.

Evaporation & Water Retention



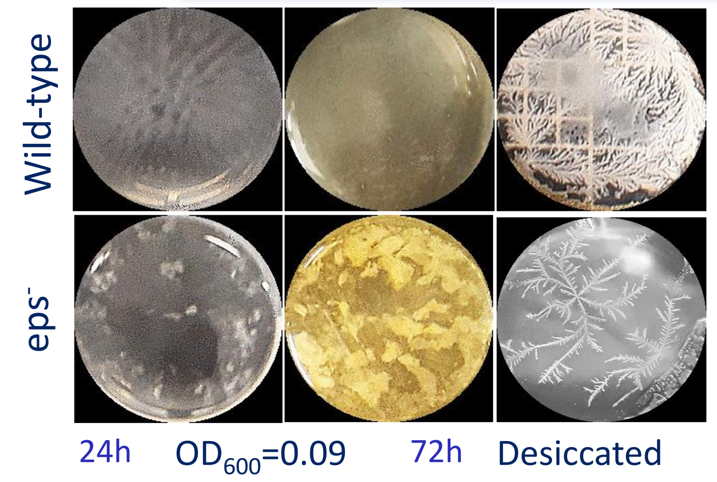


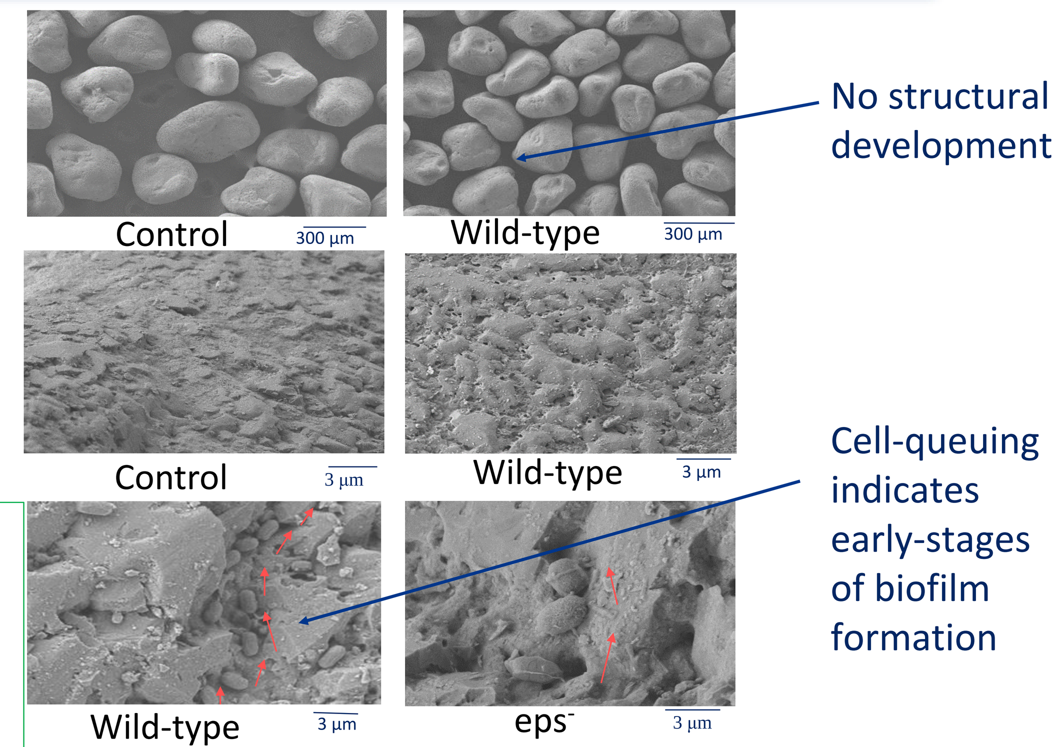




- Higher critical drying front depth (L_c) in the control than in the treatment columns;
- Less cumulative evaporation from the treatments;
- No distinct evaporation phase transition between phase I and phase II;
- Wild-type strain-treated sand retained the most water towards the drier end of the WRC (from 15% water content).

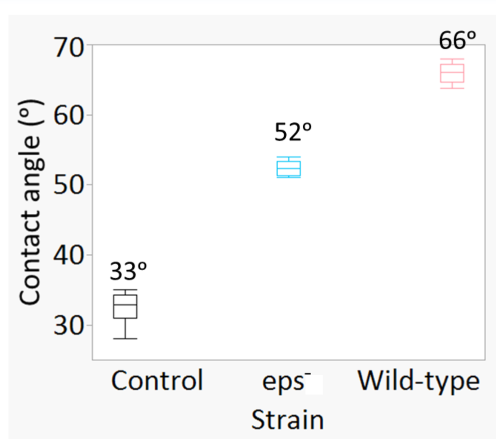
Pellicle Formation & SEM Imaging

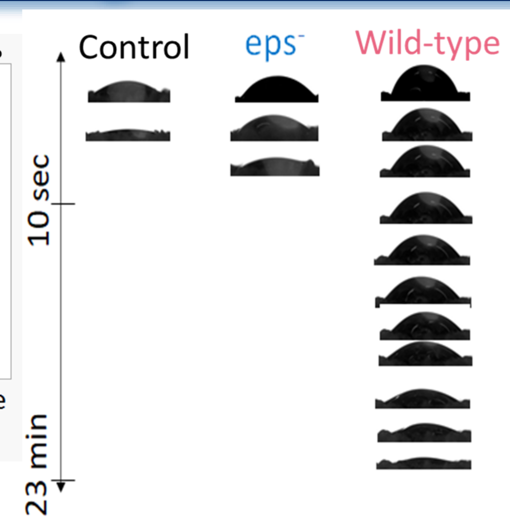


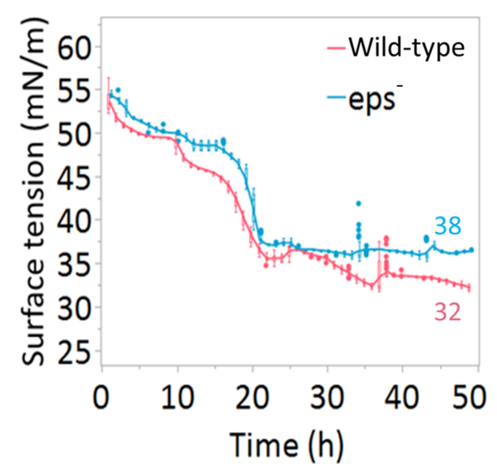


- Wild-type variant formed dendritic, continuous pellicles;
- eps⁻ formed deformed, discrete pellicles; due to activation of other related genes of *eps* operon;
- Wrinkled, channelized topology of the Wild-type pellicle could be hydrophobic (Werb et al., 2011).

Contact Angle and Surface Tension

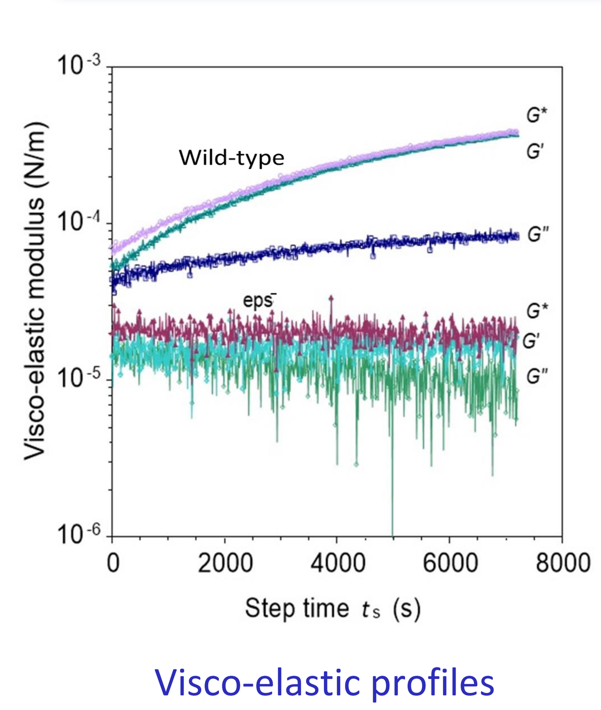




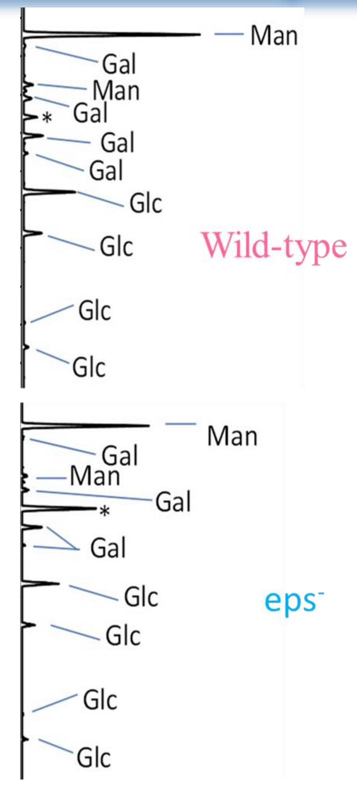


- Both strains increased contact angle of treated sand and reduced surface tension of LB solution;
- Water droplet persistency showed reduced wetting or hydraulic stabilization by the wild-type.

Viscosity & EPS Composition



Visco-elastic profiles



Carbohydrate compositions

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

Possible Mechanisms

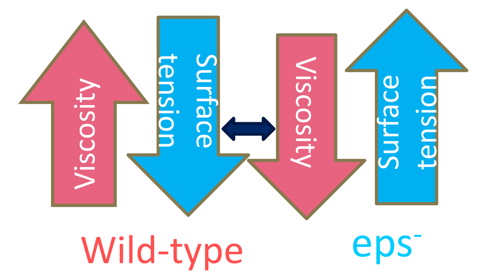
- Increased viscosity or viscous dissipation length (L_V) lowered evaporative loss by reducing the critical drying front depth (L_c):

$$L_C = \frac{L_G}{\frac{L_G}{L_V}+1}$$ (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:

$$h = \frac{-2\sigma\cos\theta}{r\rho g}$$

- The dampening effect of viscosity maintained the connectedness at the drier end (occurrence of Rayleigh instability in the presence of EPS at low Reynold's number).
- The interplay between surface tension and visco-elasticity led to complex changes in water retention and flow in treated sand.



Conclusions

- Both bacterial strains increased water retention of a fine sand during both upward (evaporation) and downward (percolation) 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 PGPRs mediate changes in soil water status.

References

Lehmann, P., S. Assouline, & Or, Dani (2008). Characteristic lengths affecting evaporative drying of porous media. *Phys. Rev. E Stat. Nonlin. Soft Matter Phys.* 77:056309. doi:10.1103/PhysRevE.77.056309

Werb, M., García, C.F., Bach, N.C., et al (2017). Surface topology affects wetting behavior of *Bacillus subtilis* biofilms. *npj Biofilms Microbiomes 3:0–1*. doi: 10.1038/s41522-017-0018-1