Acinetobacter baylyi Evolution and Microbial Engineering

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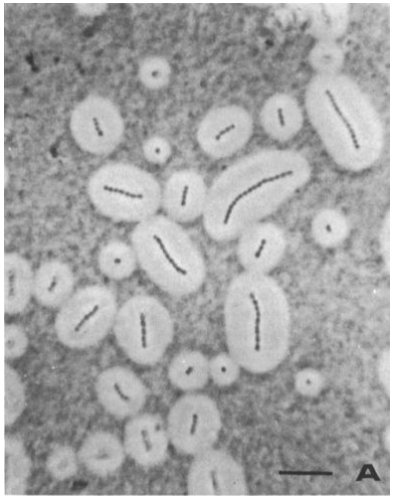
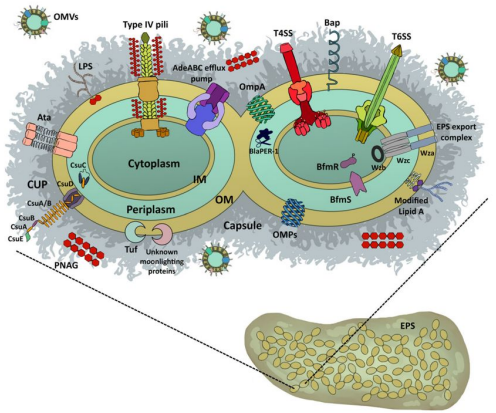
Acinetobacter baylyi

- Useful for studying evolution
  - Naturally competent at transformation (integrate external DNA into chromosome)
- Wild type clumps together → capsule layer (slime)
- Mutated strain w/o capsule clumps together → surface proteins (>20)
  - Individually removing and testing would take years



Wild type (BD4 strain)



Mutated to remove capsule

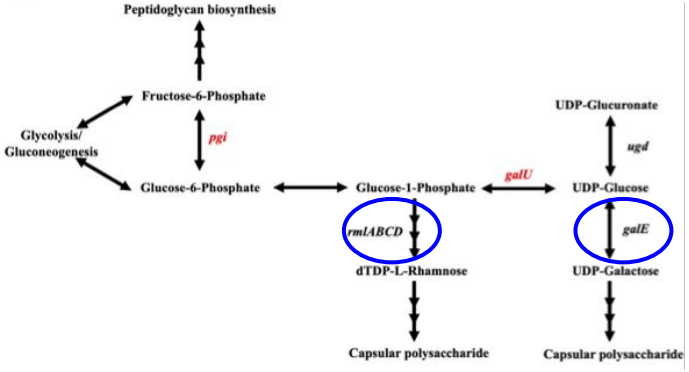
Goal: use evolution make strain without capsule and without specific surface proteins that make it clump

Preparing for Evolution Experiment

- Made 2 strains that removed genes for capsule synthesis
  - Δrml strain (single knockout)
  - Δrml/ΔgalE strain (double knockout)

- PCR to amplify DNA before/after gene we want to remove
- Use natural competence of *A. baylyi* to integrate these flanks (with gene removed) into its genome

Synthesis Pathway for Capsule (polysaccharide)

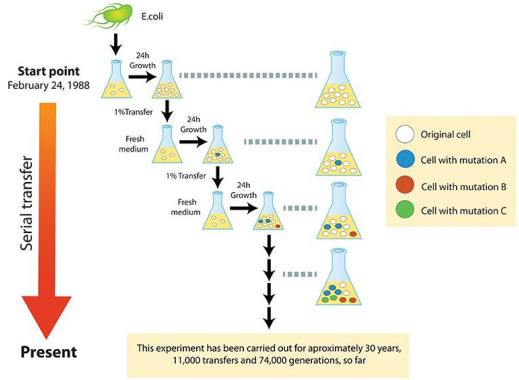


Evolution Experiment

- Grow 24 populations over 30 days
  - Six populations of 4 strains
  - 300 generations - 9,000 years on a human scale
- Evolutionary pressure to not clump: centrifugation (heavy clumps sink)
- Serial transfer: 50 uL from top of centrifuge tube into new media every 24 hrs

- Evidence evolution is occurring: turbidity of tubes after centrifugation
  - Cells are evolving to not clump together by removing surface proteins
- Next step: sequence DNA to determine proteins removed through evolution

Long Term Evolution Experiment



Day 17 populations 4 and 6