

Microbes and Math 2019



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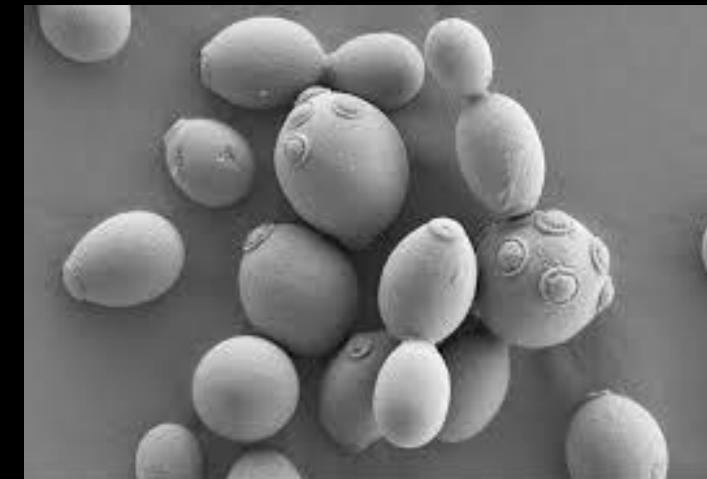
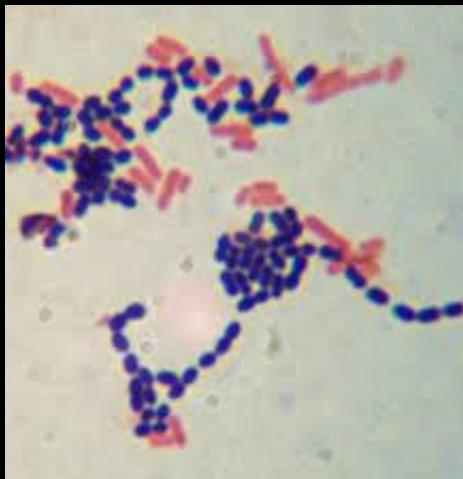
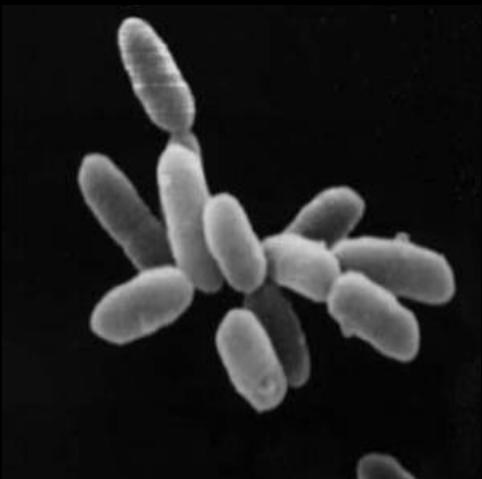
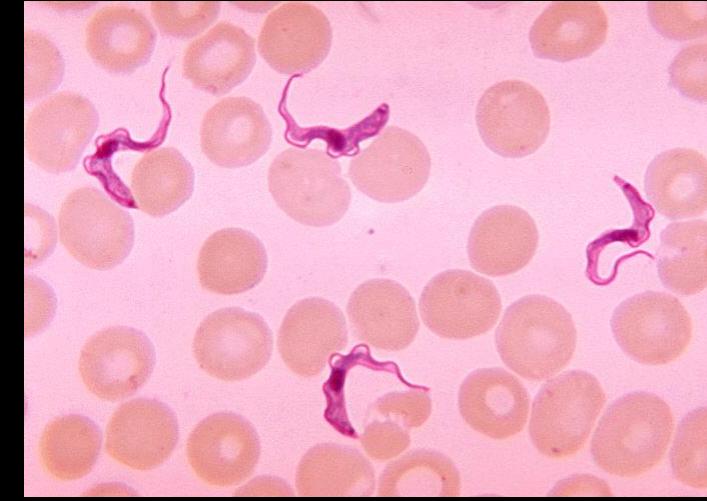
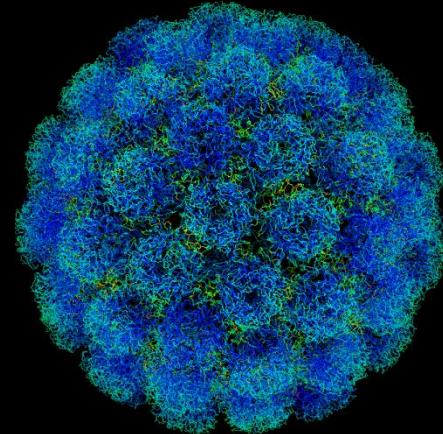


OUTLINE

- What are microbes?
 - Microbes in the environment – and in us!
- Microbes in the Schmid Lab!
- Basics of Microbial growth
 - Technologies and techniques for microbiology
- **Bio Break**
- Thinking about and planning Friday's experiment

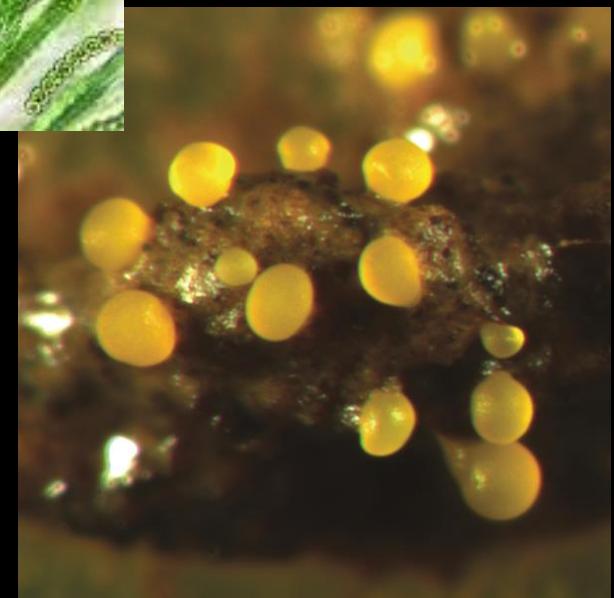
MICROBES

- Unicellular eukaryotes
- Fungi
- Bacteria
- Archaea
- Viruses



WHAT ROLE DO MICROORGANISMS PLAY IN THE ENVIRONMENT?

- Oxygen production!
 - cyanobacteria in the ocean
- Nitrogen fixation
 - symbionts with many plants
- Decompose dead material
- Help animals digest food

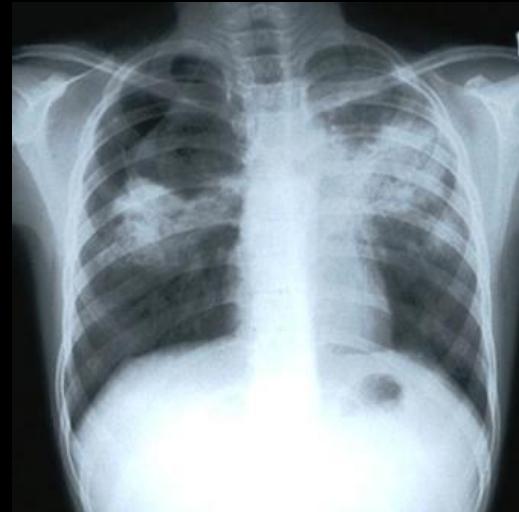


HOW DO WE USE MICROORGANISMS?

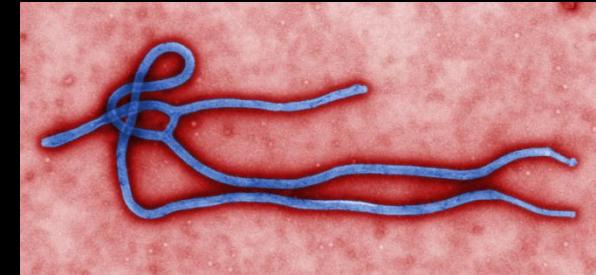
Product	Microorganism
Cheese	bacteria, fungi and milk
Alcoholic beverages	bacteria, yeast and sugar
Soy sauce	fungi and soybeans
Vinegar	bacteria and sugar
Yogurt	bacteria and milk
Sour cream	bacteria and cream
Artificial sweetener	bacteria and sugar
Bread	yeast and dough
Antibiotics	bacteria, fungi
Human Growth Hormone, human Insulin	genetically engineered bacteria
laundry enzymes	bacteria
vitamins	bacteria

INFECTIOUS DISEASES IN THE NEWS

- MRSA
 - Methicillin Resistant *Staphylococcus aureus*
- VRE
 - Vancomycin Resistant *Enterococcus*
- VRSA
 - Vancomycin Resistant *Staphylococcus aureus*
- MDR-TB
 - Multidrug Resistant Tuberculosis



- Malaria
 - *Plasmodium* parasite
- Toxoplasmosis
 - *Toxoplasma* parasite
- Zika virus
- Ebolavirus



THE HUMAN

Bacteria, fungi, and viruses outnumber human cells in the body by a factor of 10 to one. The microbes synthesize key nutrients, fend off pathogens and impact everything from weight gain to perhaps even brain development. The Human Microbiome Project is doing a census of the microbes and sequencing the genomes of many. The total body count is not in but it's believed over 1,000 different species live in and on the body.

**25
SPECIES**

in the **stomach** include:

- *Helicobacter pylori*
- *Streptococcus thermophilus*

**500-
1,000
SPECIES**

in the **intestines** include:

- *Lactobacillus casei*
- *Lactobacillus reuteri*
- *Lactobacillus gasseri*
- *Escherichia coli*
- *Bacteroides fragilis*
- *Bacteroides thetaiotaomicron*
- *Lactobacillus rhamnosus*
- *Clostridium difficile*

MICROBIOME

**600+
SPECIES**

in the **mouth, pharynx and respiratory system** include:

- *Streptococcus viridans*
- *Neisseria sicca*
- *Candida albicans*
- *Streptococcus salivarius*

**1,000
SPECIES**

in the **skin** include:

- *Pityrosporum ovale*
- *Staphylococcus epidermidis*
- *Corynebacterium jeikeium*
- *Trichosporon*
- *Staphylococcus haemolyticus*

**60
SPECIES**

in the **urogenital tract** include:

- *Ureaplasma parvum*
- *Corynebacterium aurimucosum*

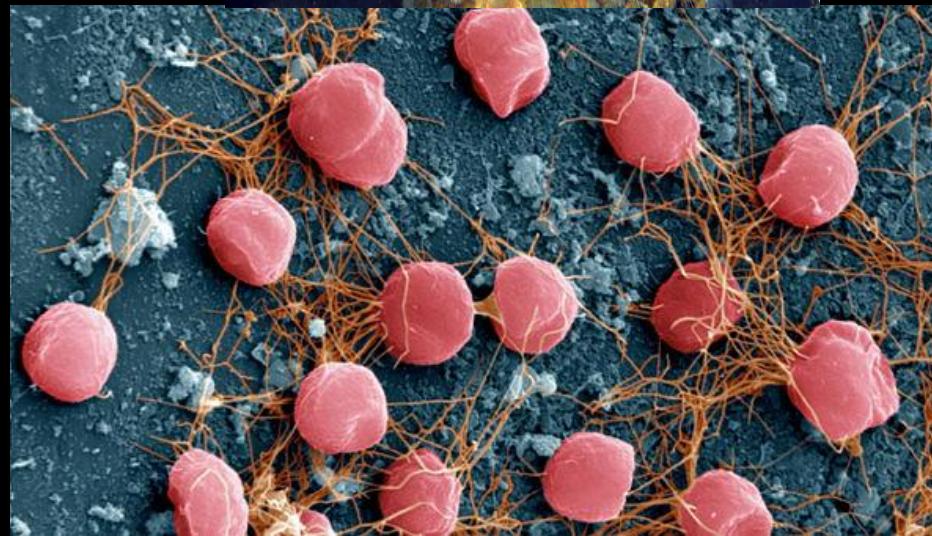
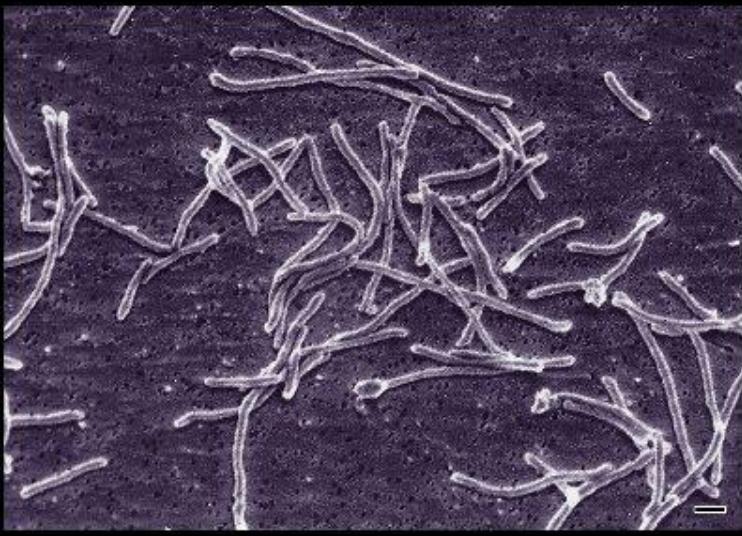
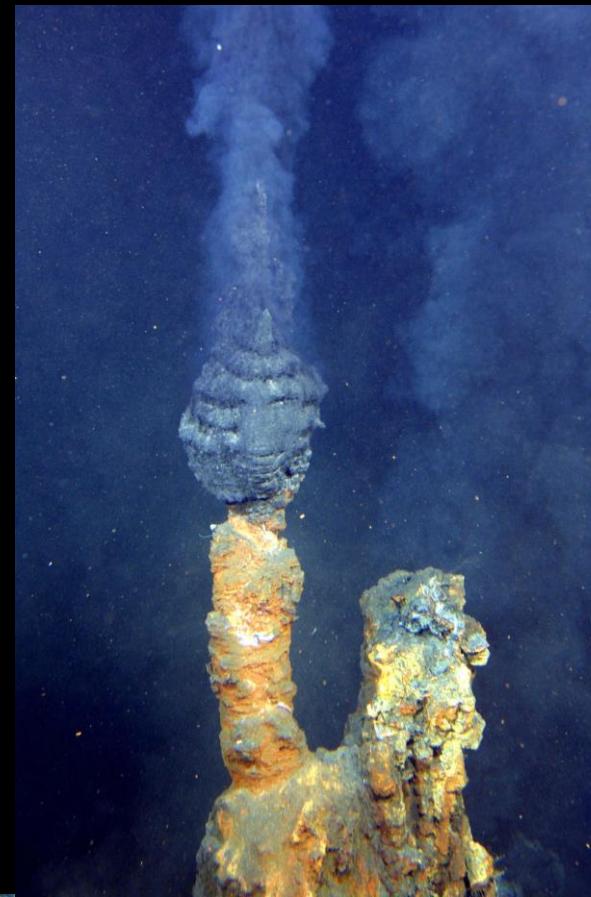


WHAT IS AN EXTREMOPHILE?

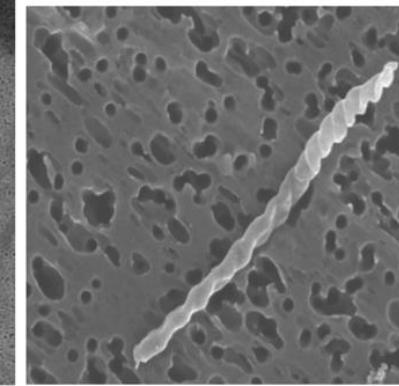
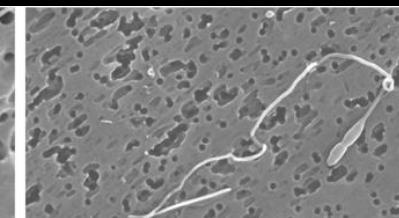
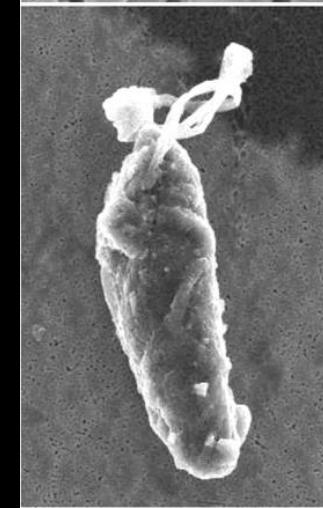
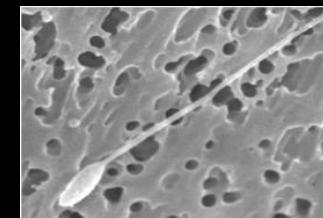
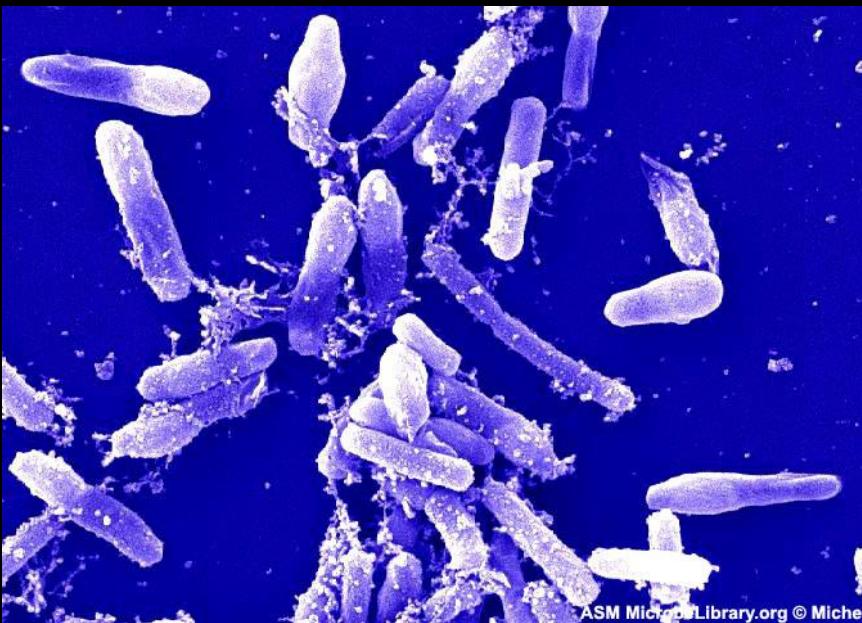
Extremophiles are organisms that thrive in very extreme environments - they can survive conditions that would kill most other organisms!



THERMOPHILES



PSYCHROPHILES



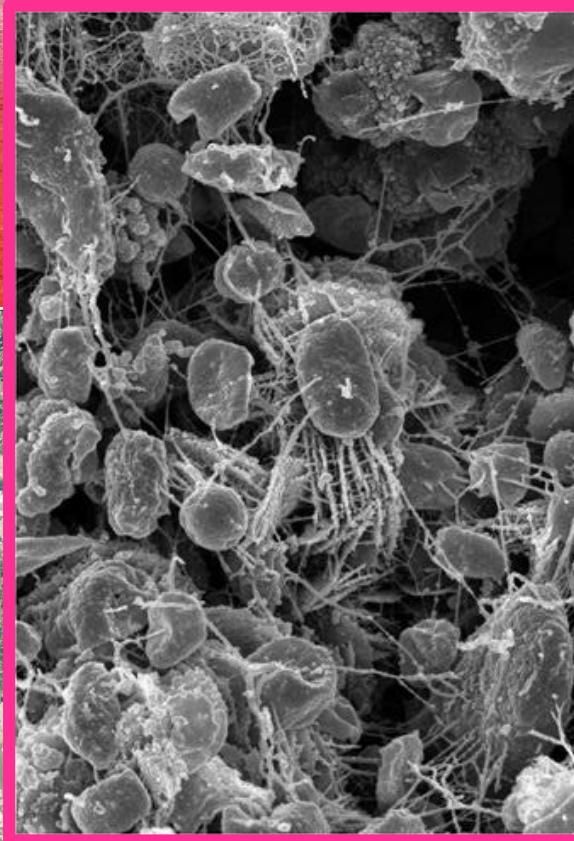
ASM Microbe Library.org © Michel

ACIDOPHILES

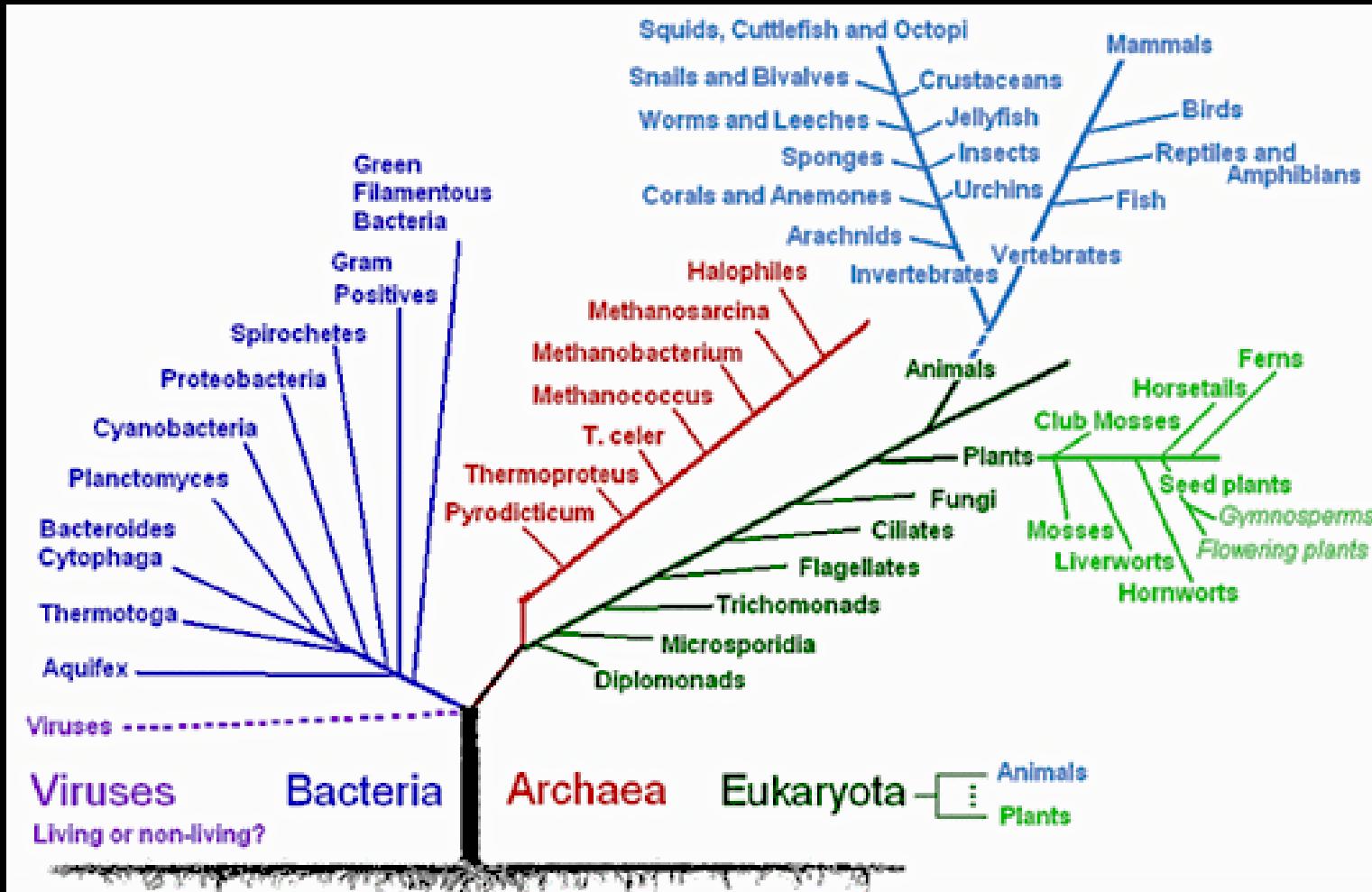


Massive growth of the extreme acidophile
Ferroplasma in a California mine.

HALOPHILES



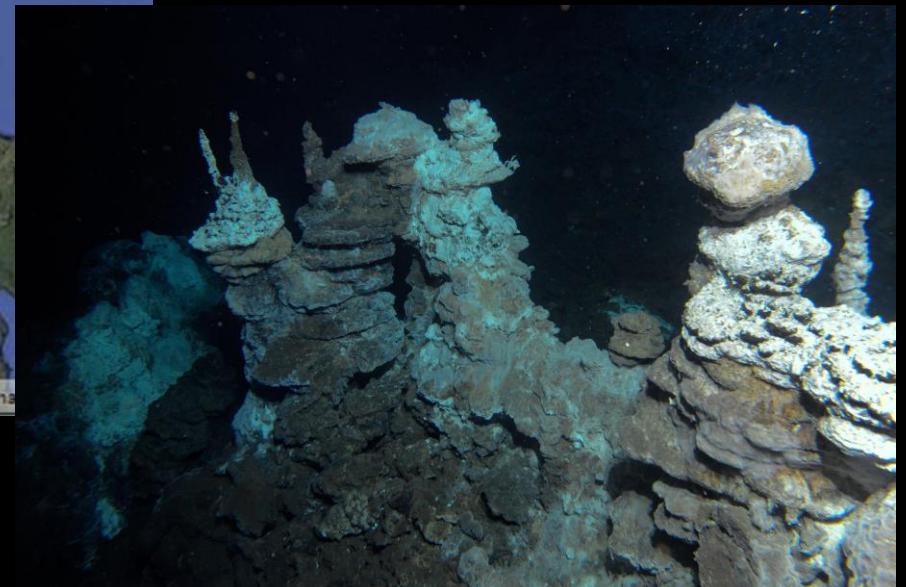
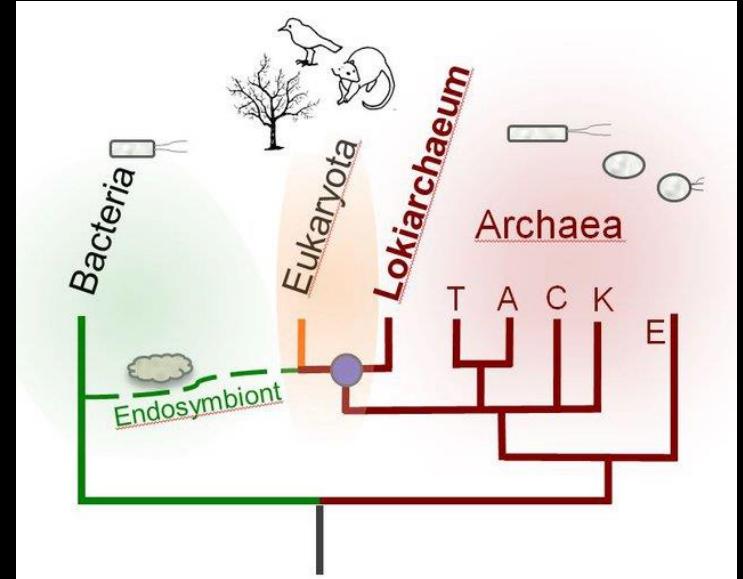
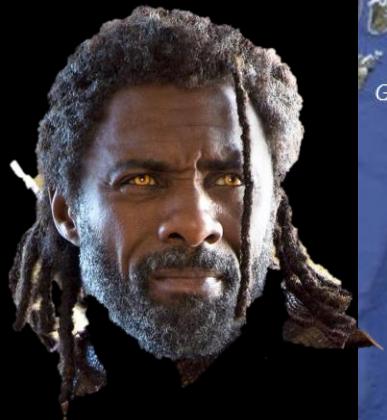
The tree of life

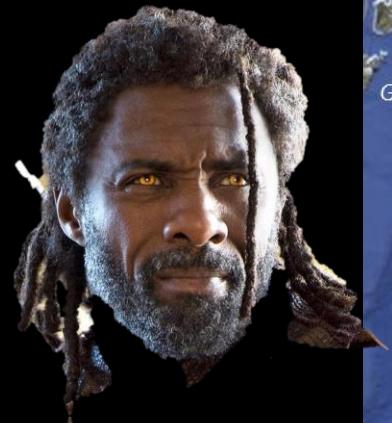


Why Archaea?

diversity
extremophiles
medical applications
our close cousins
our microbiota

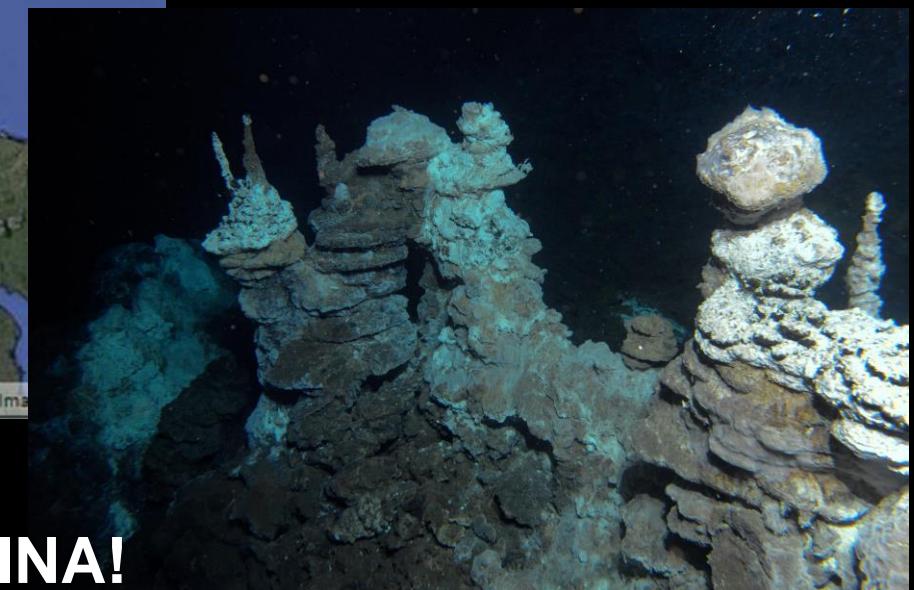
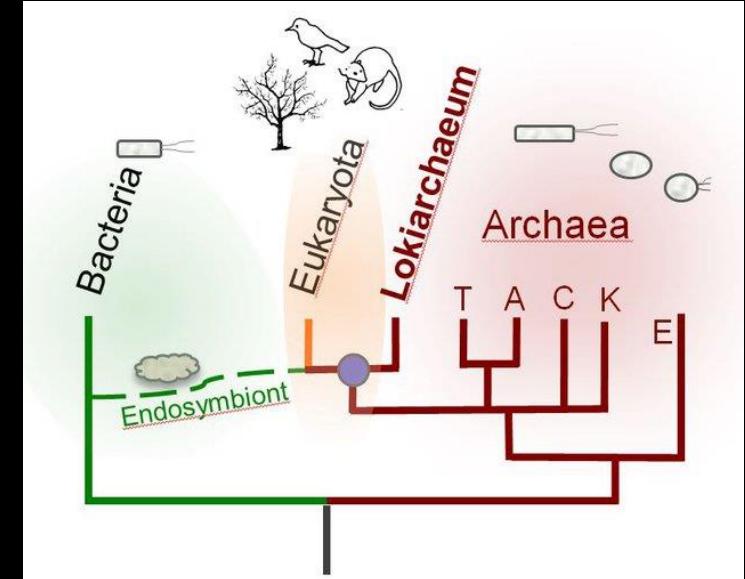
Archaea: the Asgard

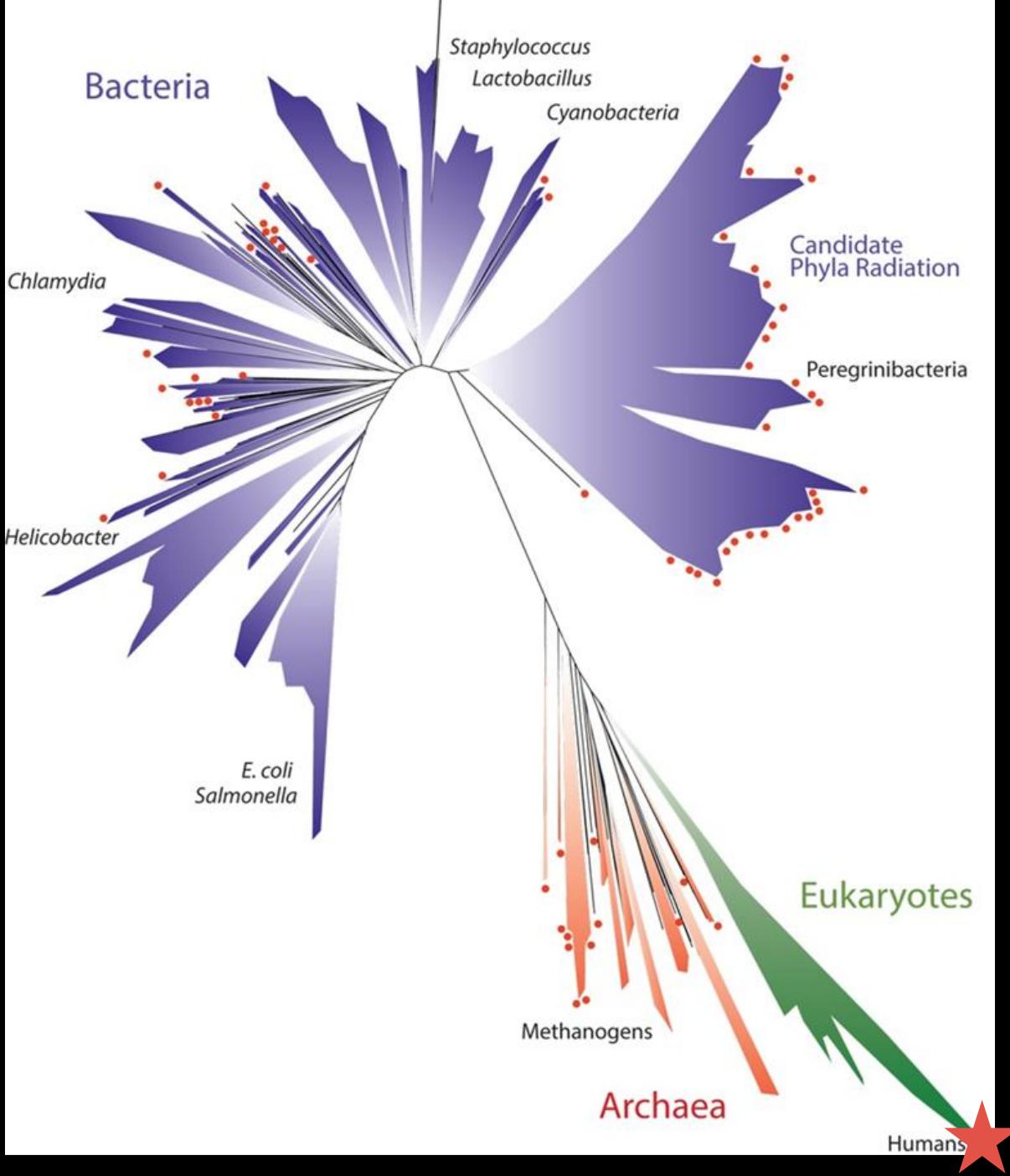




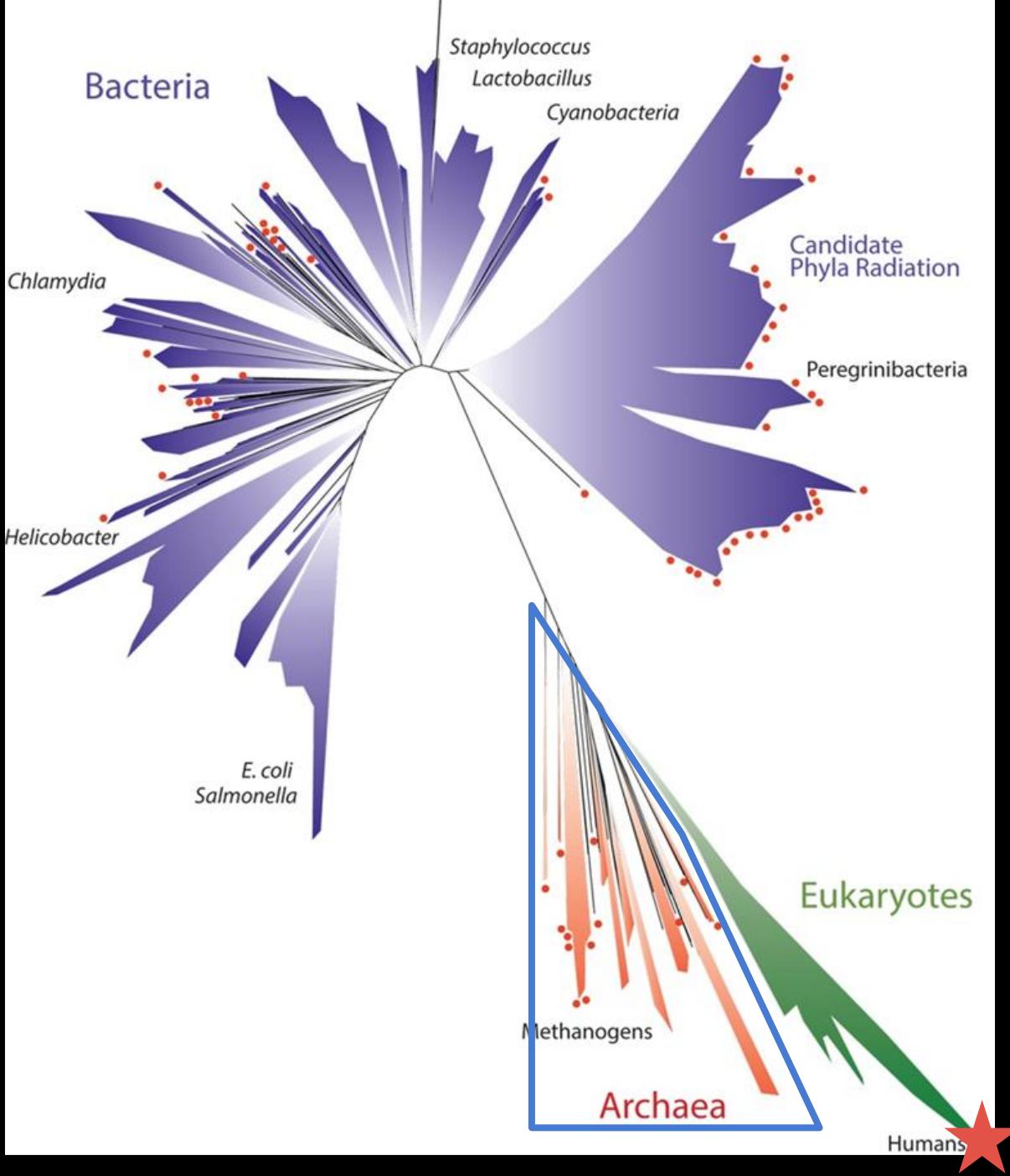
Archaea: the Asgard

DISCOVERED IN NORTH CAROLINA!

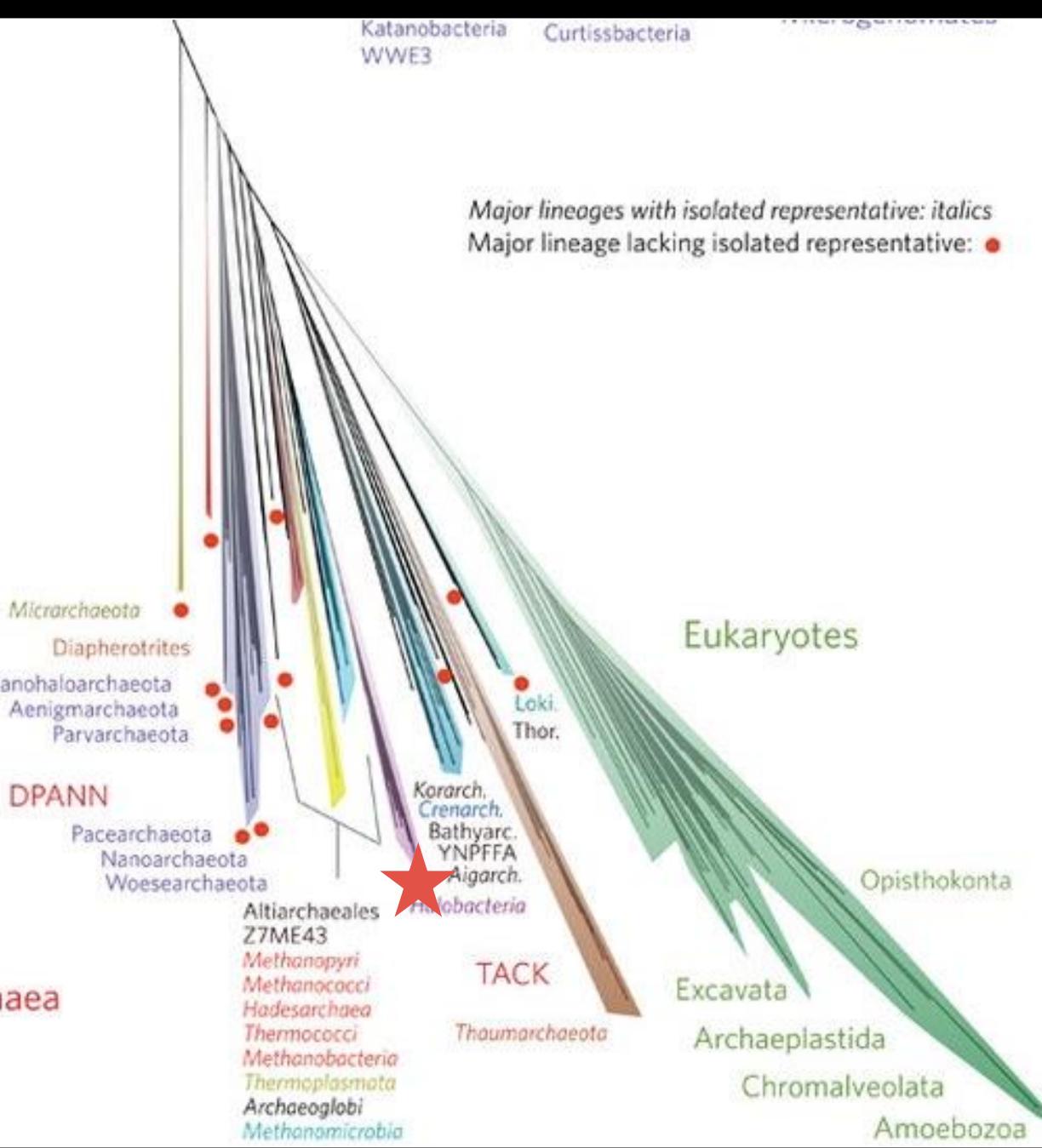




Adapted from Hug et al., 2016,
Nature Micro



Adapted from Hug et al., 2016,
Nature Micro



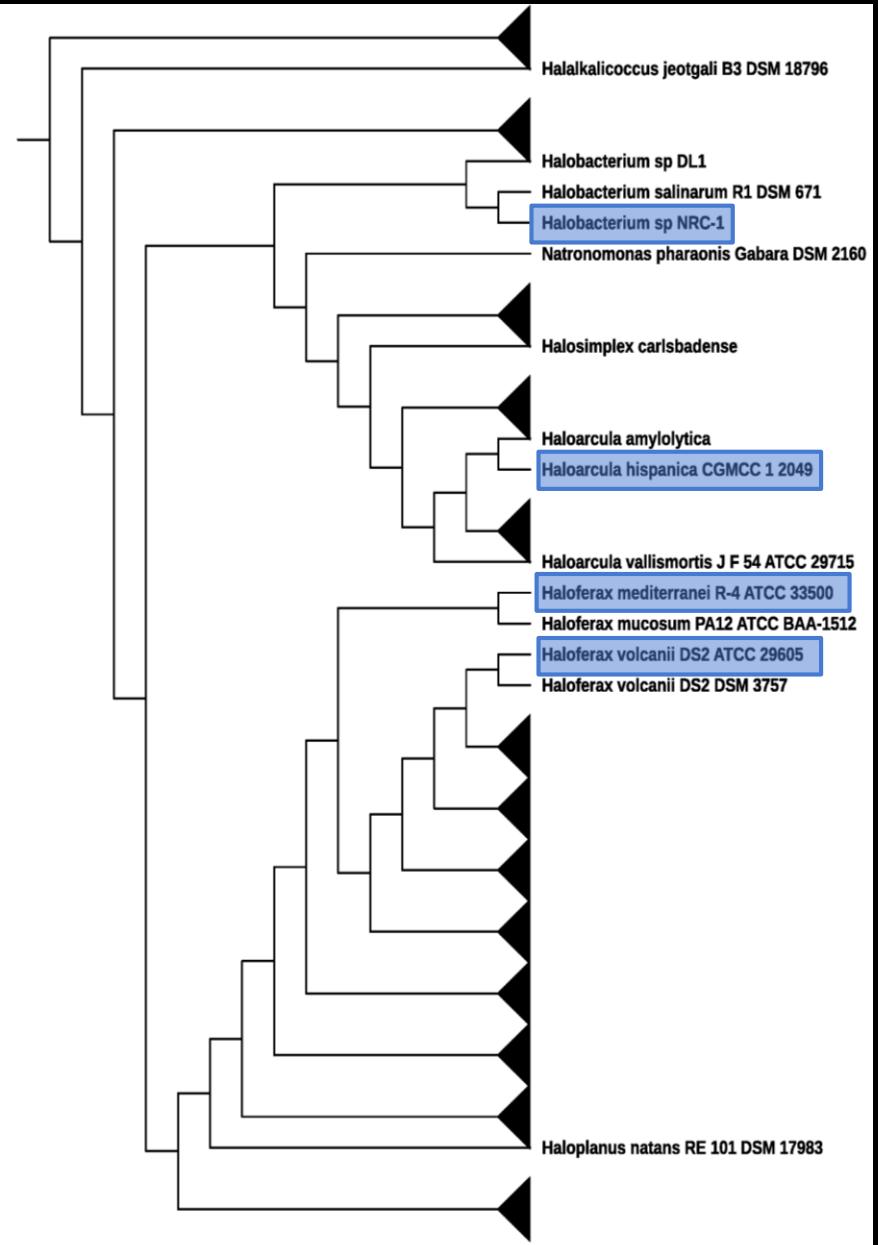
Halobacterium salinarum is model archaeon



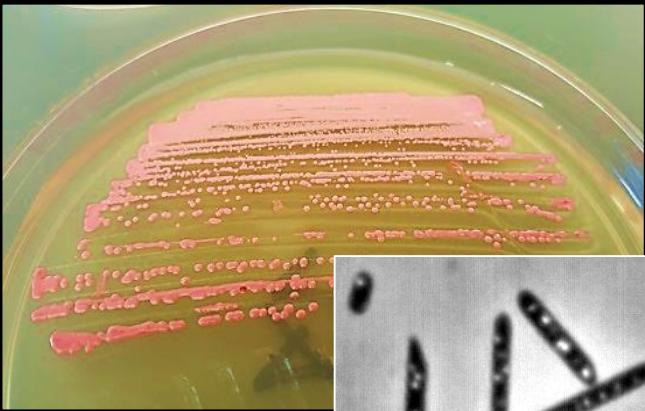
Great Salt Lake (photo:Brent Watson)



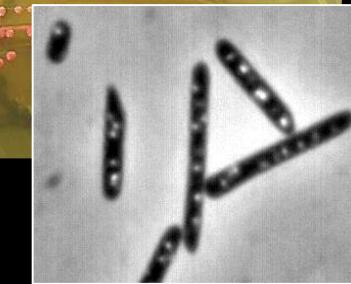
MEET OUR “BUGS”



Halobacterium salinarum



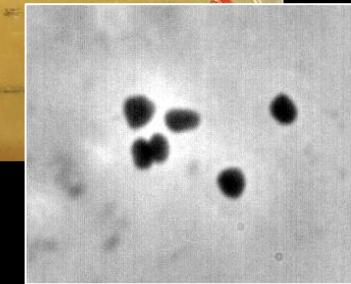
4.2M NaCl



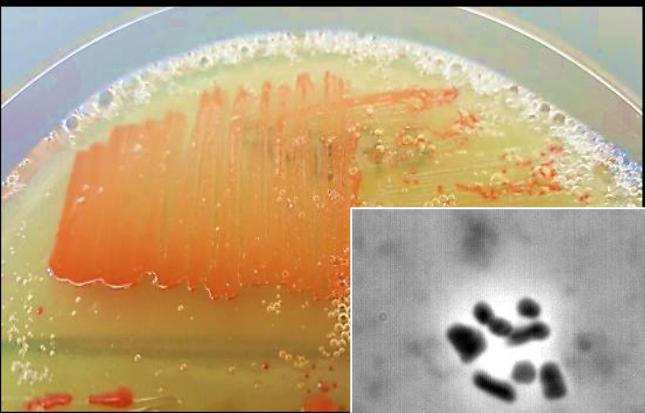
Haloferax volcanii



2.5M NaCl



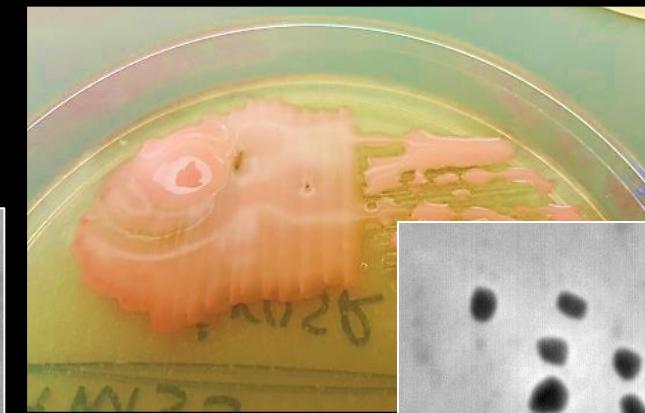
Haloarcula hispanica



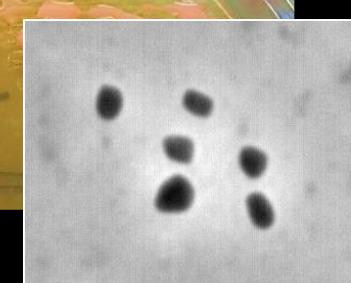
3.4M NaCl



Haloferax mediterranei

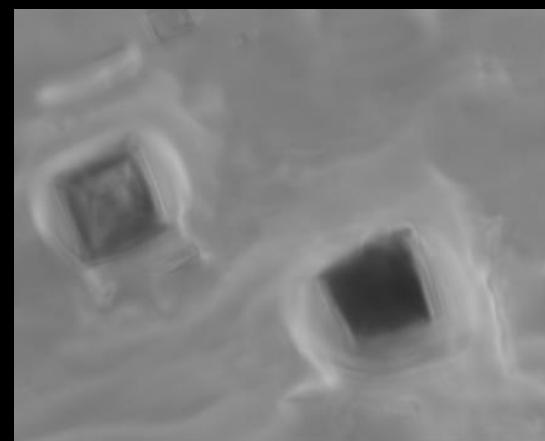
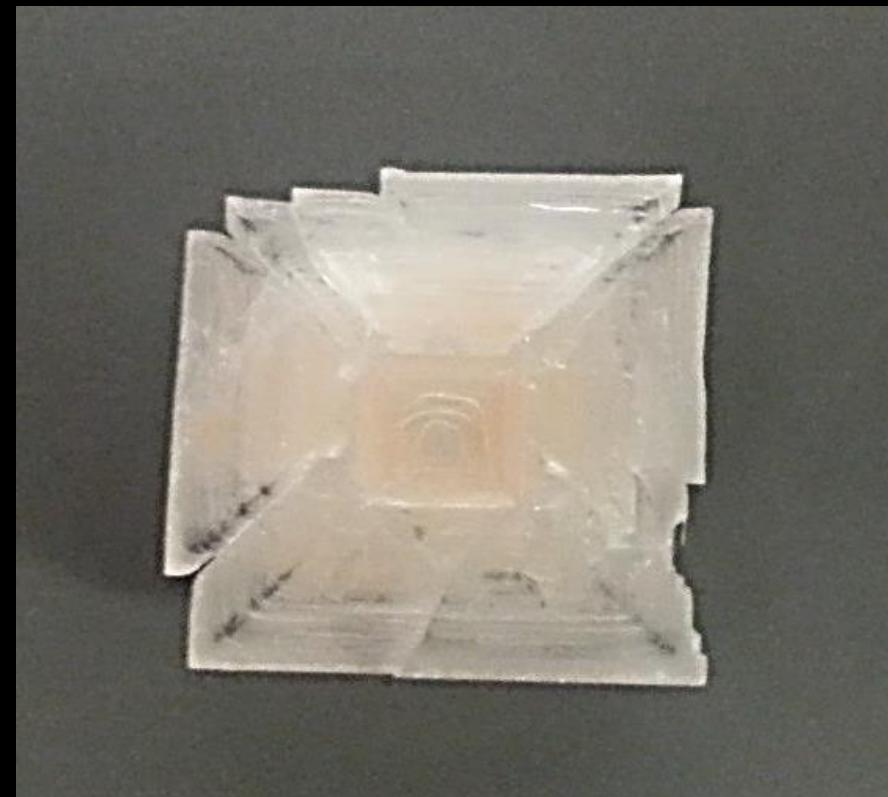
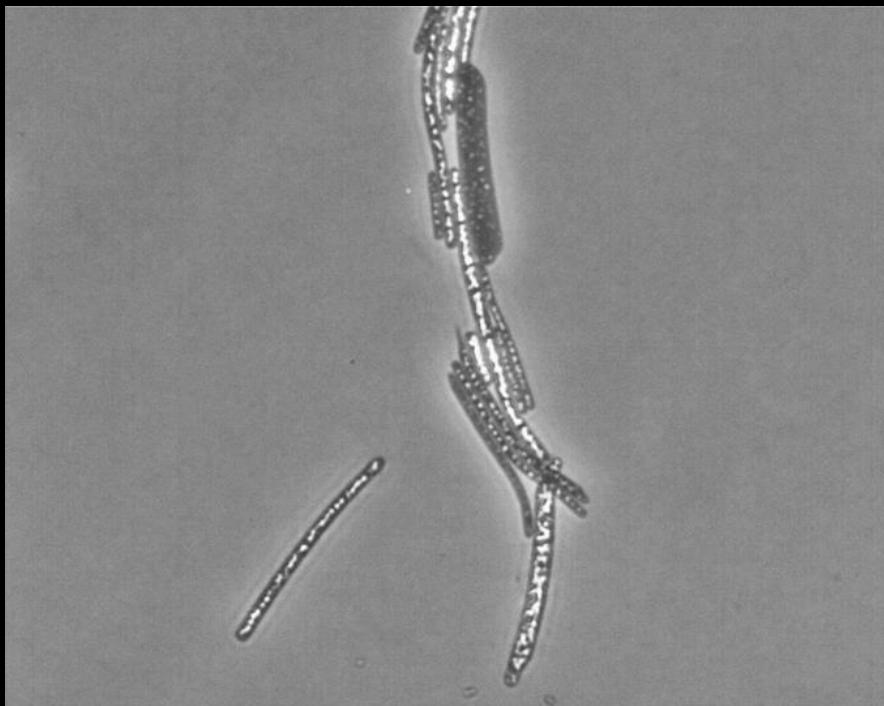


2.5M NaCl



Fun facts about *Halobacterium salinarum*

- Prefers 4.2 M NaCl, 42°C for optimal growth
- Survives UV-C at 254nm (lethal to *E. coli*)
- Survives space-like vacuum (1 millitorr)



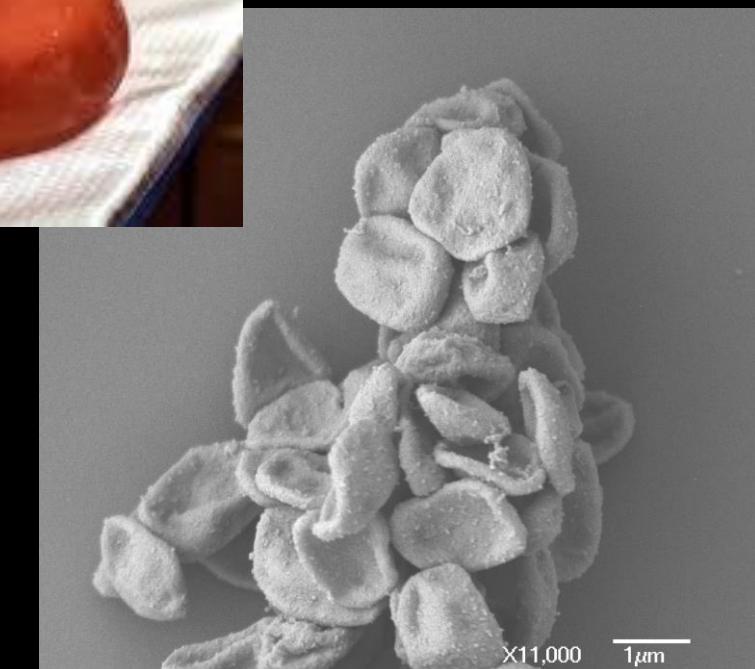
Fun facts about *Haloferax volcanii*

- Prefers 2.5 M NaCl, 45°C for optimal growth
- Isolated from the Dead Sea
- Grows faster than *H. salinarum*



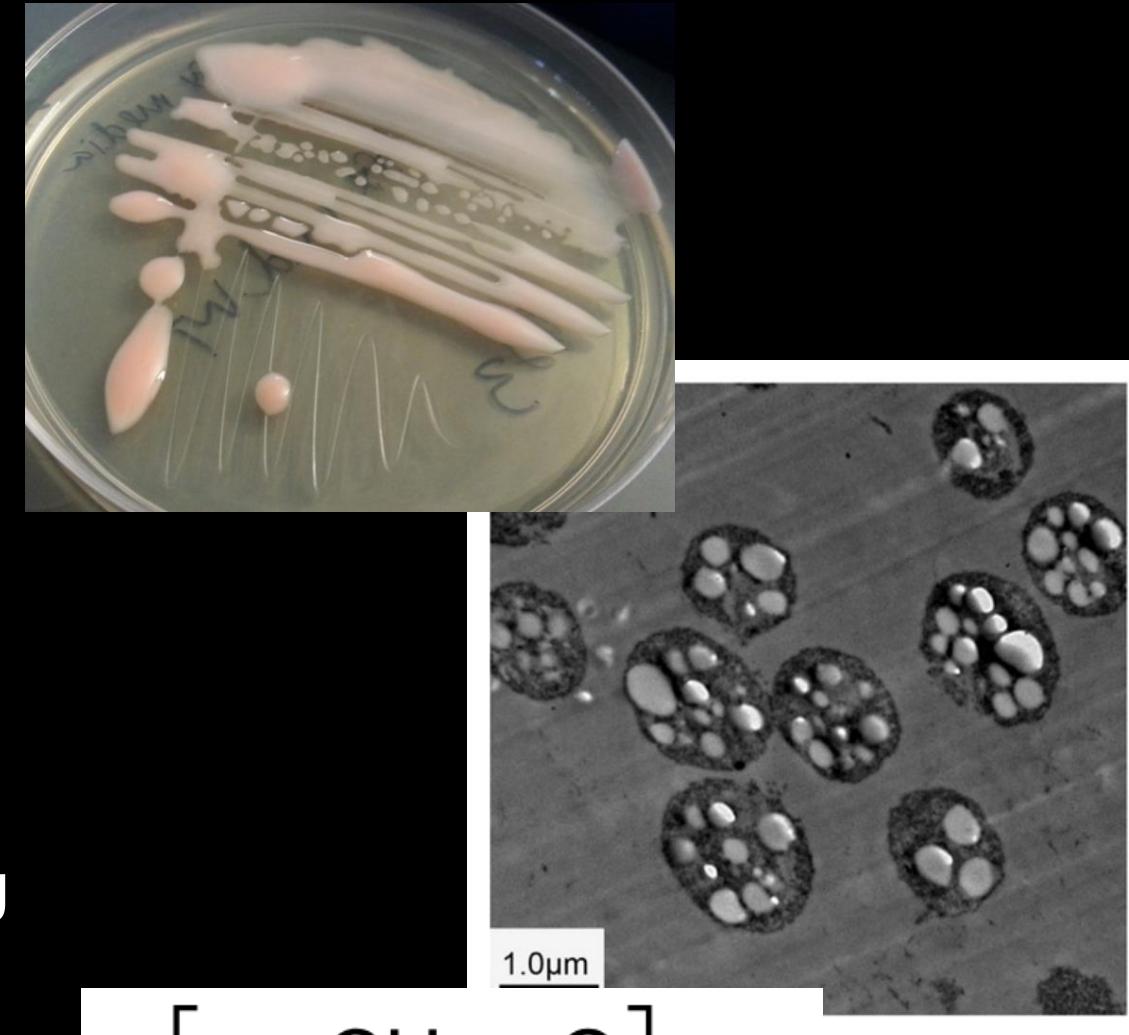
Not fun facts about *Haloferax volcanii*

- Named for microbiologist Benjamin Elazari Volcani and has nothing to do with a volcano



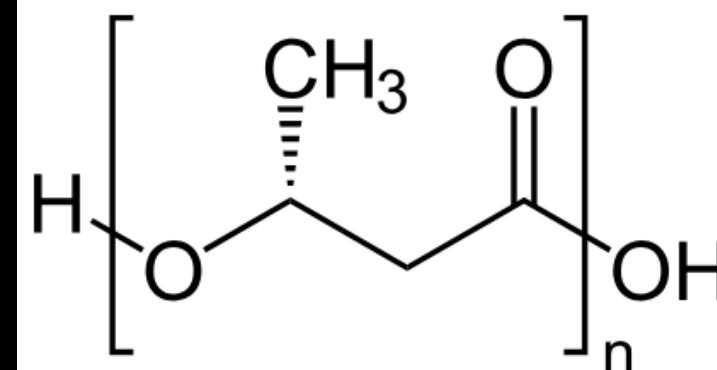
Fun facts about *Haloferax mediterranei*

- Prefers 2.5 M NaCl, 45°C for optimal growth
- Isolated from Alicante, Spain
- Grows faster than *H. salinarum* and *H. volcanii*
- Makes PHA (polyhydroxyalkanoates) – used to make biodegradable bioplastics
- First archaeal species identified as having CRISPRs (1993)



Not fun facts about *Haloferax mediterranei*

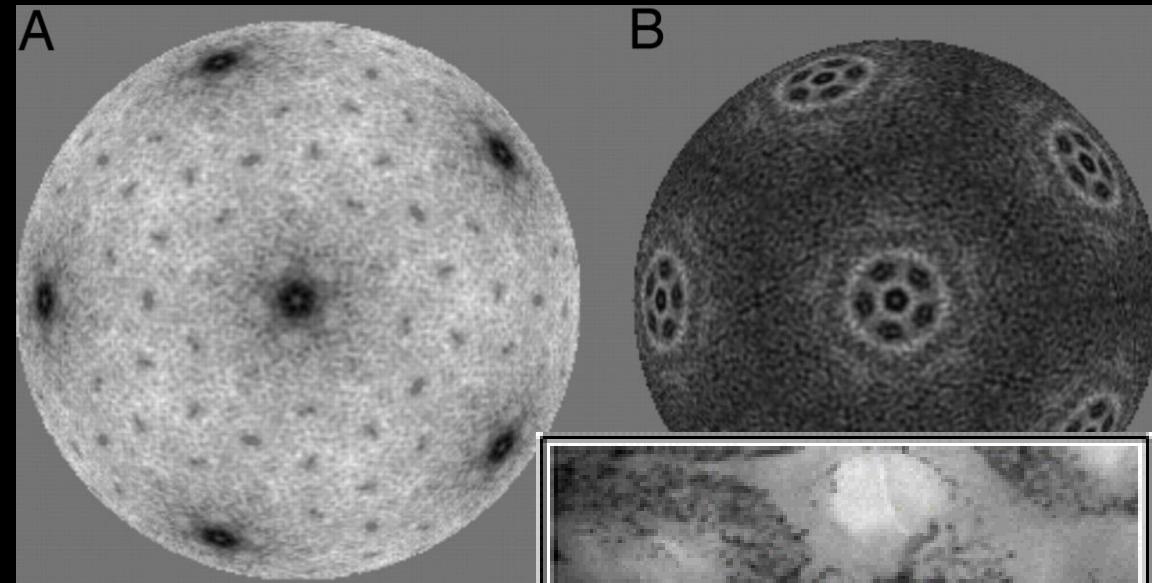
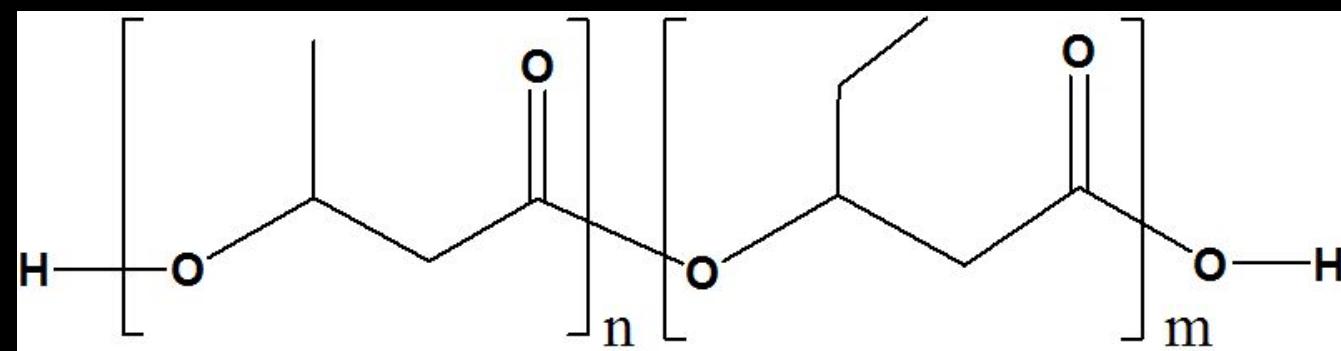
- Smells horrible



Cai et al 2012,
JBact

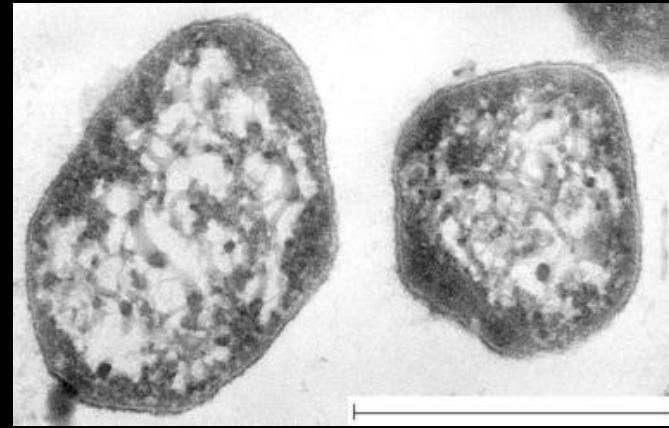
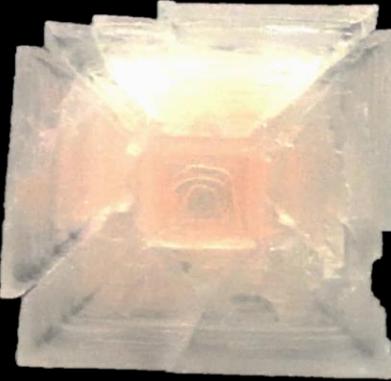
Fun facts about *Haloarcula Hispanica*

- Prefers 3.9 M NaCl, 37°C for optimal growth
- Isolated from Alicante, Spain
- Grows faster than *H. salinarum*
- Initial model for Host-virus interactions in saline environments
- Makes PHBV (poly-3-hydroxybutyrate-co-3-hydroxyvalerate)— used to make biodegradable bioplastics



H. hispanica
Liu et al 2013, J Prot. Res.

Halophiles can
survive
MILLIONS of
years in salt
crystals

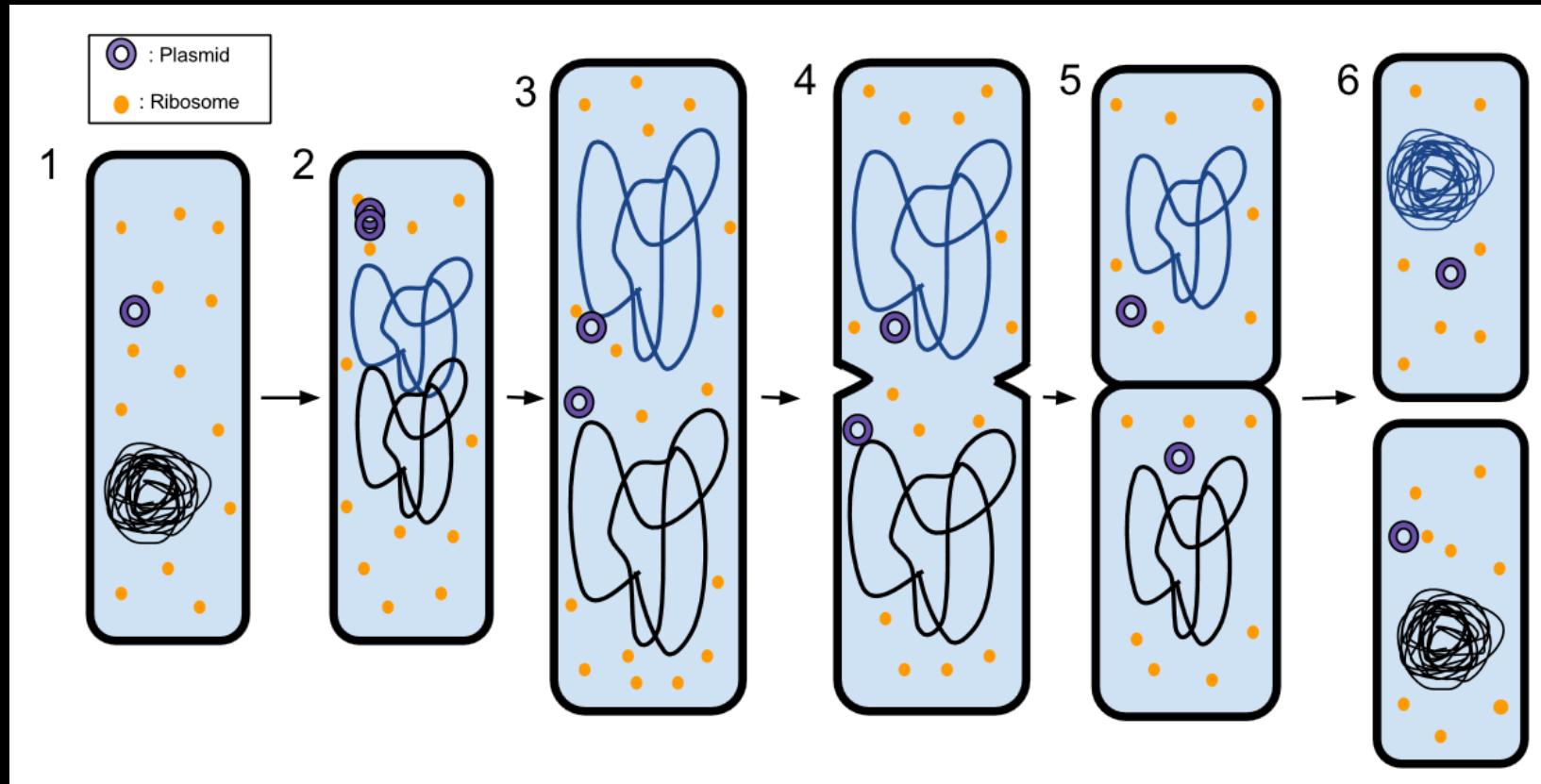


Era	Period	Stage in Reptile Evolution
Cenozoic	Tertiary 65Mya	Modern forms evolve
Mesozoic	Cretaceous 141Mya	Last dinosaurs present. Extinction of all large reptiles.
	Jurassic 202Mya	Dinosaurs dominant. Reptiles continue to radiate.
	Triassic 252Mya	Mass reptile radiation. Dinosaurs, marine reptiles, pterosaurs evolve.
Palaeozoic	Permian 280Mya	Mammal-like reptiles dominant.
	Carboniferous	First reptile evolved. Radiated from small forms.

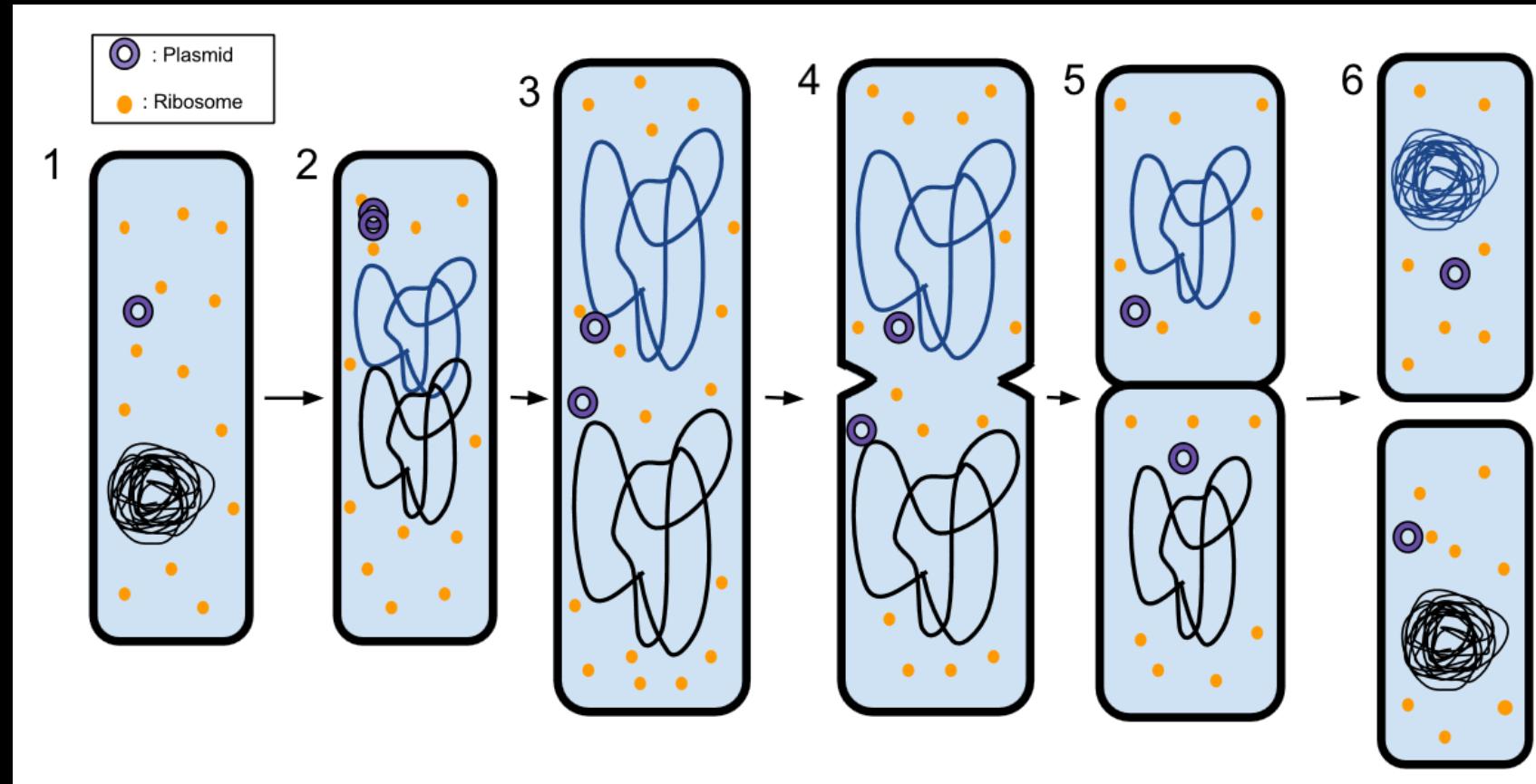


MICROBIOLOGY 101: MICROBIAL GROWTH

- Why do we study growth?

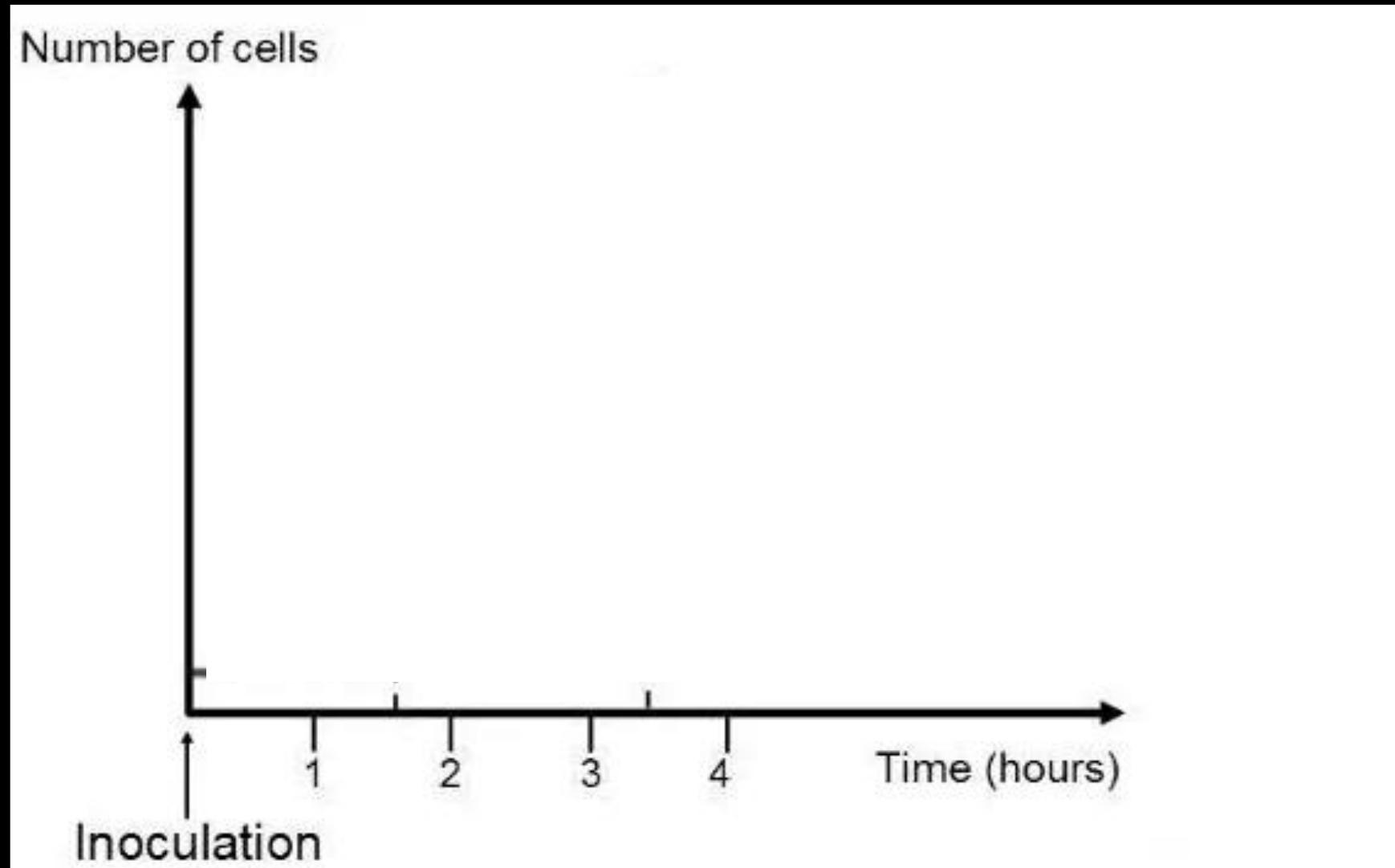


BINARY FISSION

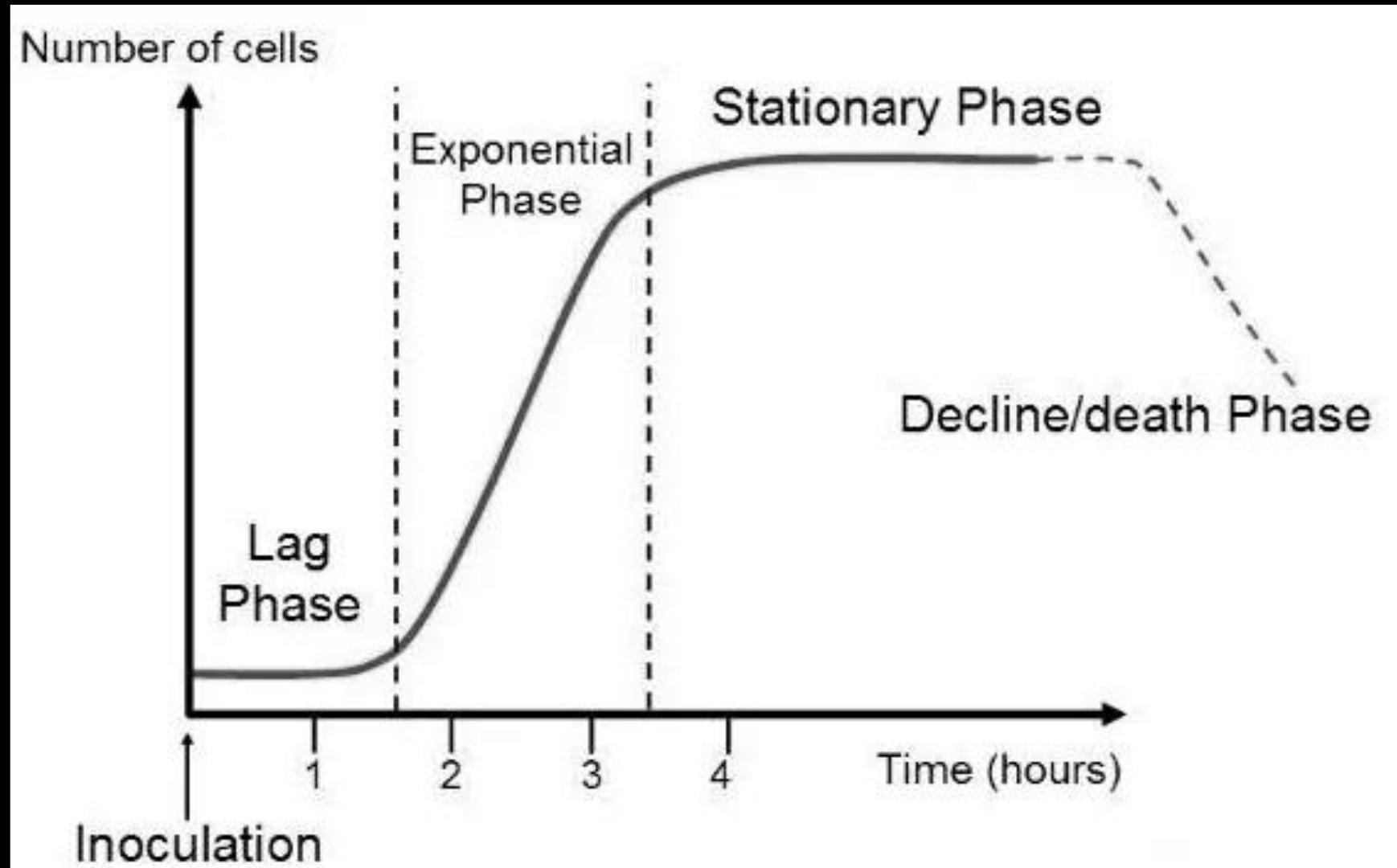


Write an equation that represents cells dividing in a population

GROWTH PHASES

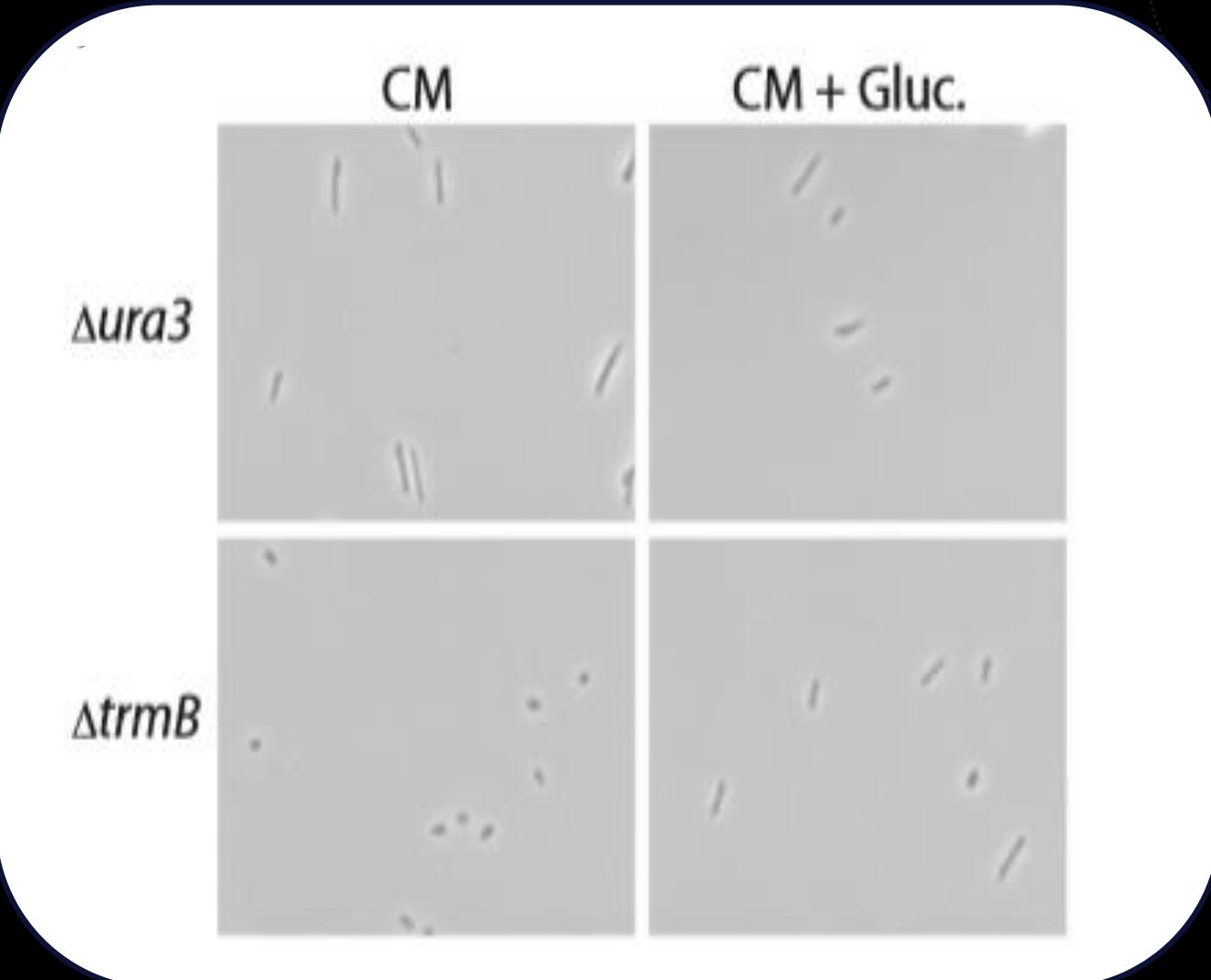


GROWTH PHASES



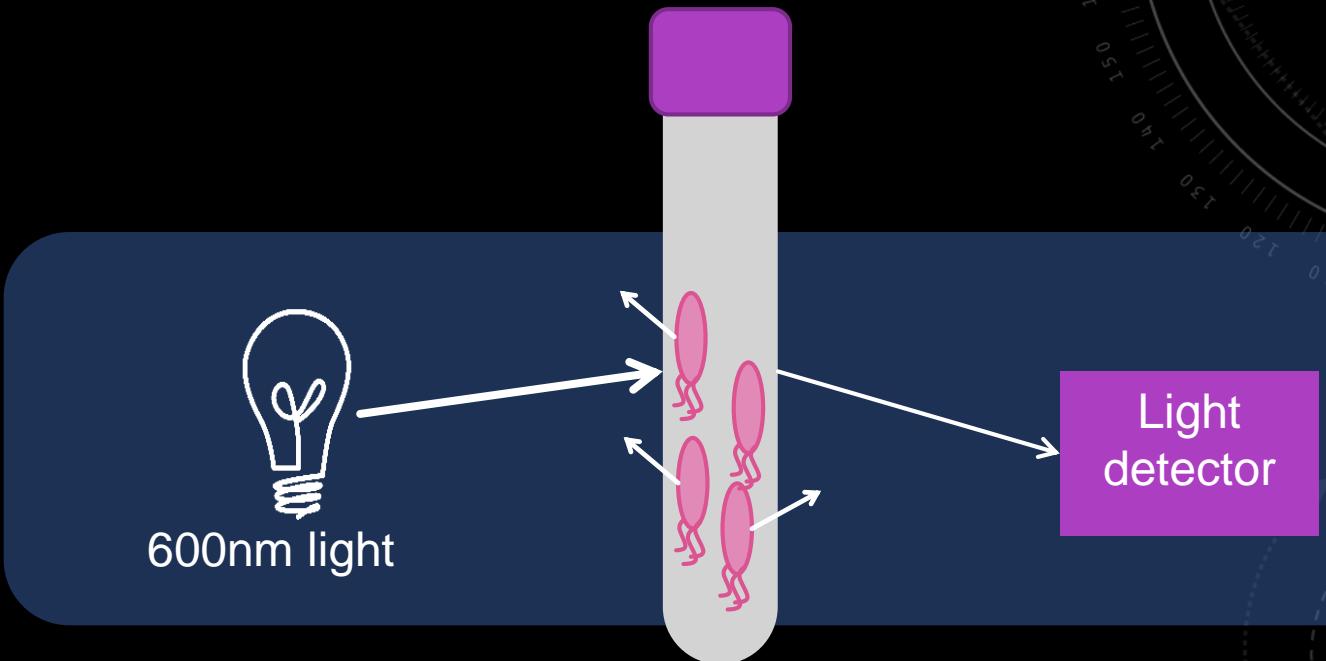
HOW CAN YOU MEASURE MICROBIAL GROWTH?

1. Microscopy counts
2. Optical density measurements
3. Counting colony forming units



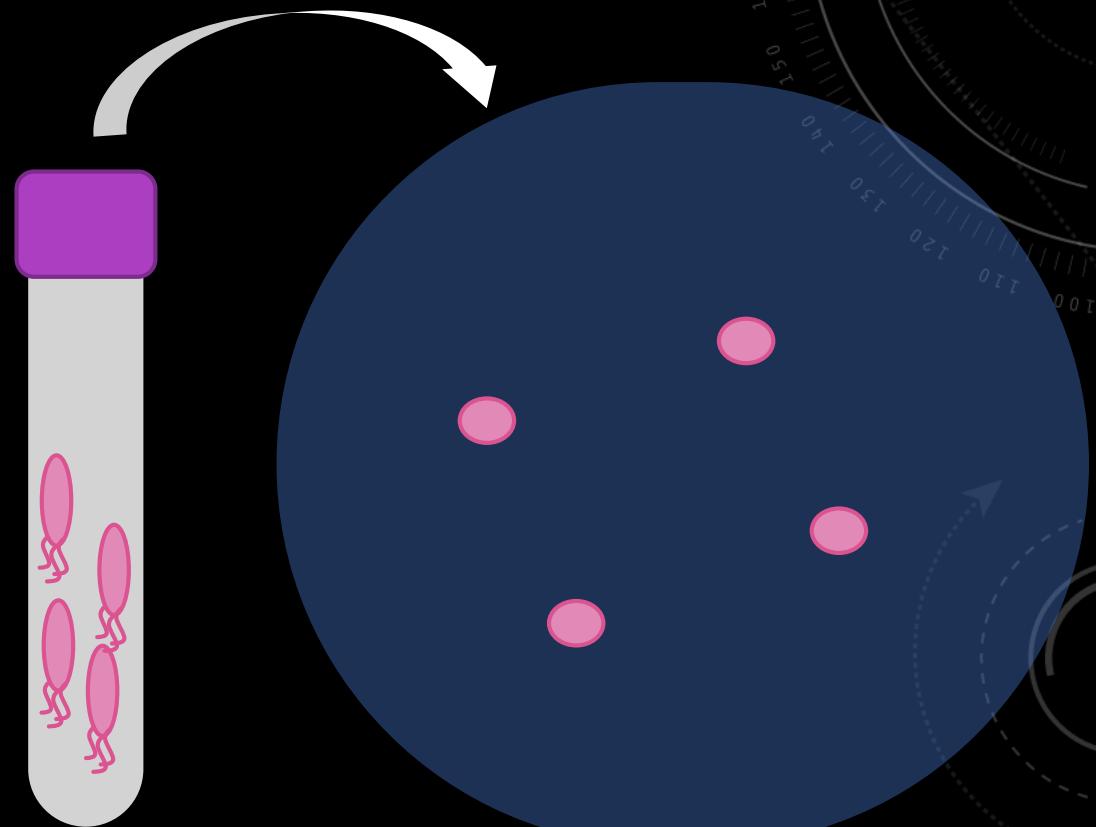
HOW CAN YOU MEASURE MICROBIAL GROWTH?

1. Microscopy counts
2. **Optical density measurements**
3. Counting colony forming units



HOW CAN YOU MEASURE MICROBIAL GROWTH?

1. Microscopy counts
2. Optical density measurement
3. **Counting colony forming units**



Incubate at 42C
for 14 days

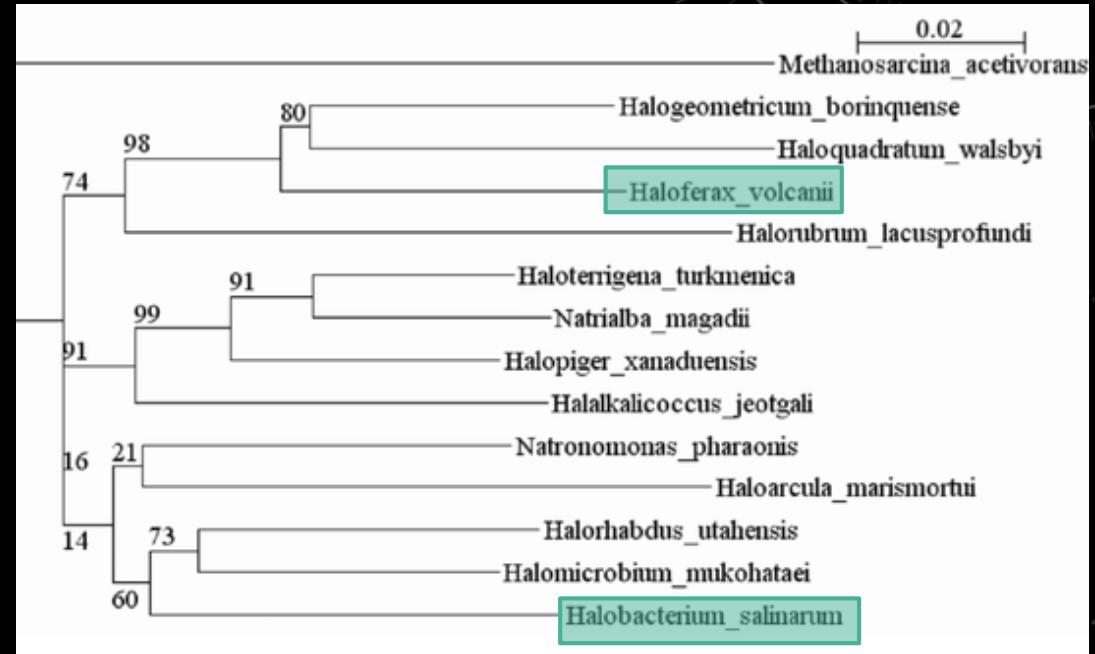
EXPERIMENTAL DESIGN

CONCLUSIONS FROM THE PAPER:

- TrmB links cell growth and metabolism in *Halobacterium salinarum*
- TrmB and its binding sequence are conserved throughout archaea
- We still don't know how cell shape, cell cycle, and growth rate are coordinated in archaea
- Combined with previous research this work paves the way for future studies on the conservation of the topology underlying this connection across all three domains.

DOES TRMB HAVE THE SAME FUNCTION IN OTHER ARCHAEA?

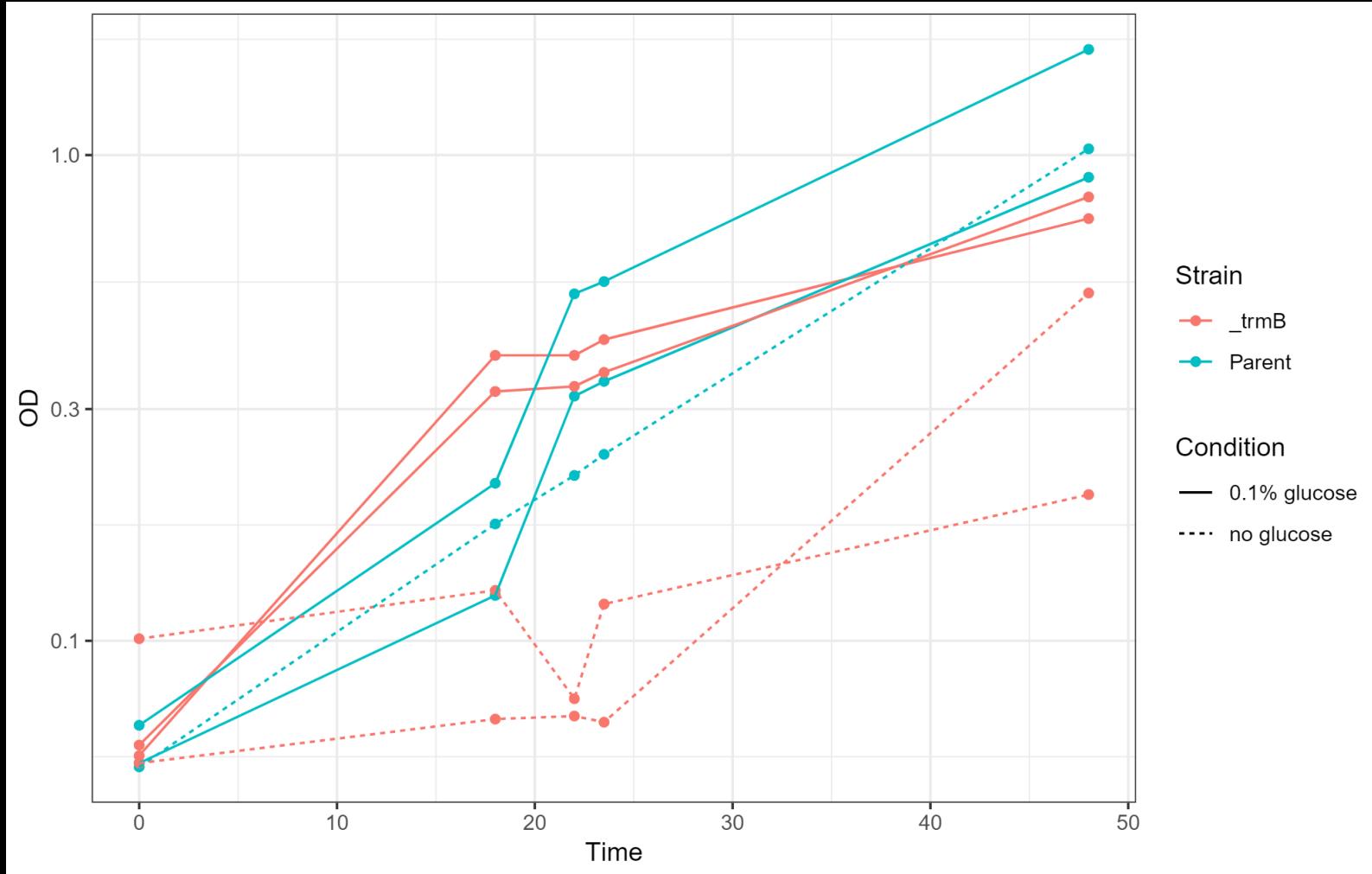
- All of this work was done in *Halobacterium salinarum*
- *Haloarcula hispanica* lives in very similar saline environments
- It last shared a common ancestor with *Hbt. salinarum* ~600 million years ago



BRAINSTORMING OUR HYPOTHESIS

- We have $\Delta trmB$ strain in *Hca. hispanica*, and media with and without sugar
- **Using methods similar to what was presented in the paper and discussed today, how could we test if $\Delta trmB$ affects cell growth in *Hca. hispanica*?**

PRELIMINARY RESULTS: $\Delta TRMB$ STRAIN IN *HCA. HISPANICA* HAS A GROWTH DEFECT

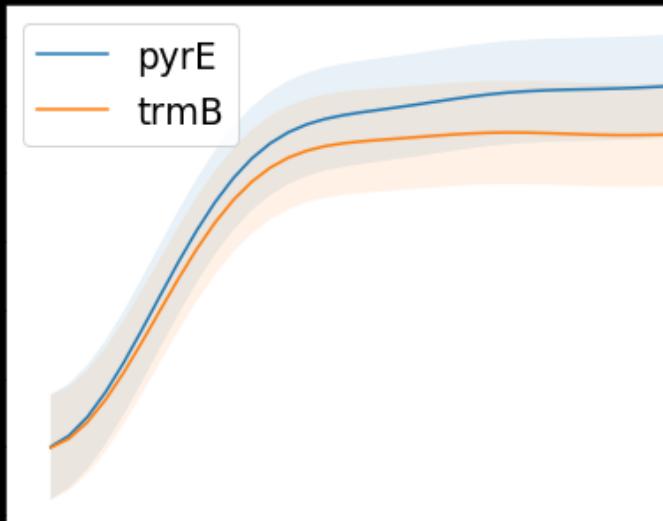


HIGH FREQUENCY GROWTH CURVE USING ROBOT

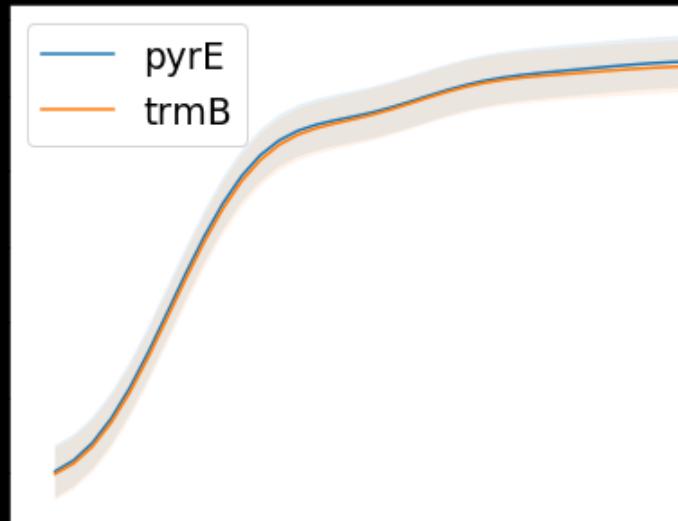
- Accepts two 100-well plates, up to 200 samples per experiment
- Constant temperature
- Shaking
- Measures OD every 30 minutes
- Measures multiple locations per well



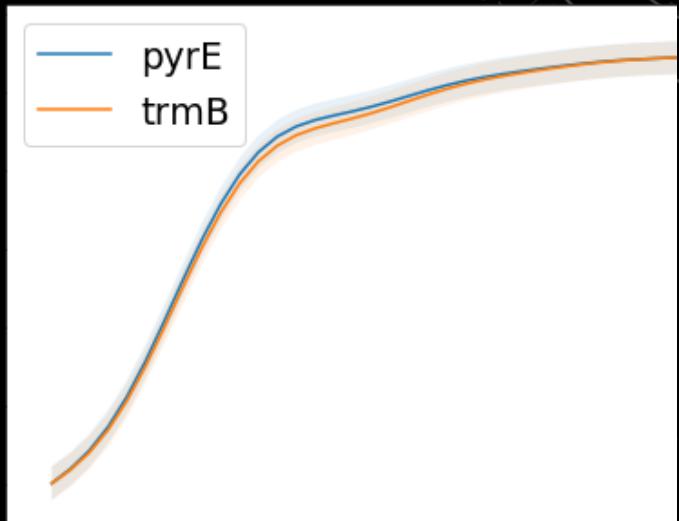
HIGH FREQUENCY GROWTH CURVE USING ROBOT



0.05% glu
Bayes: 178.3



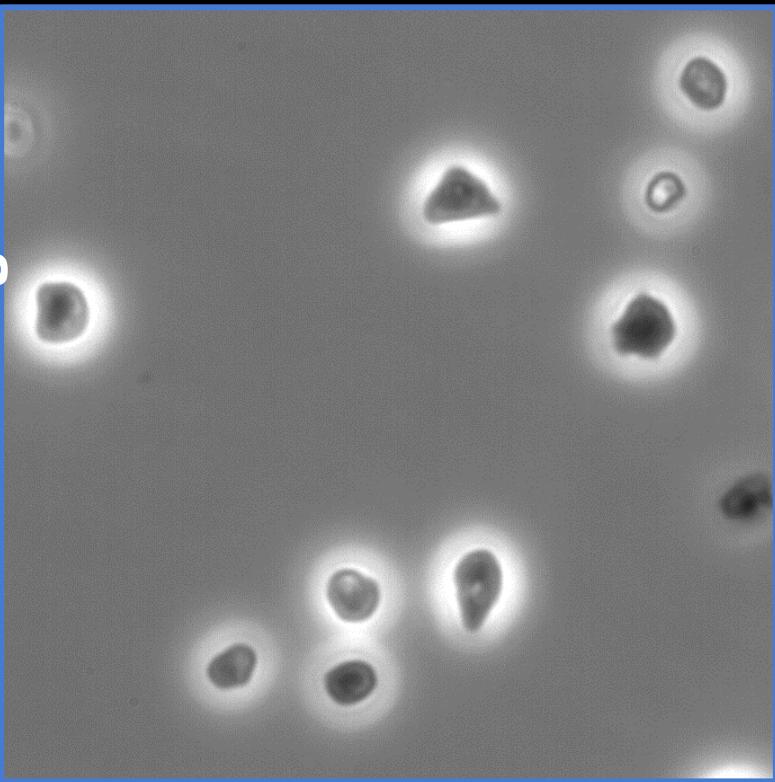
0.1% glu
9.74



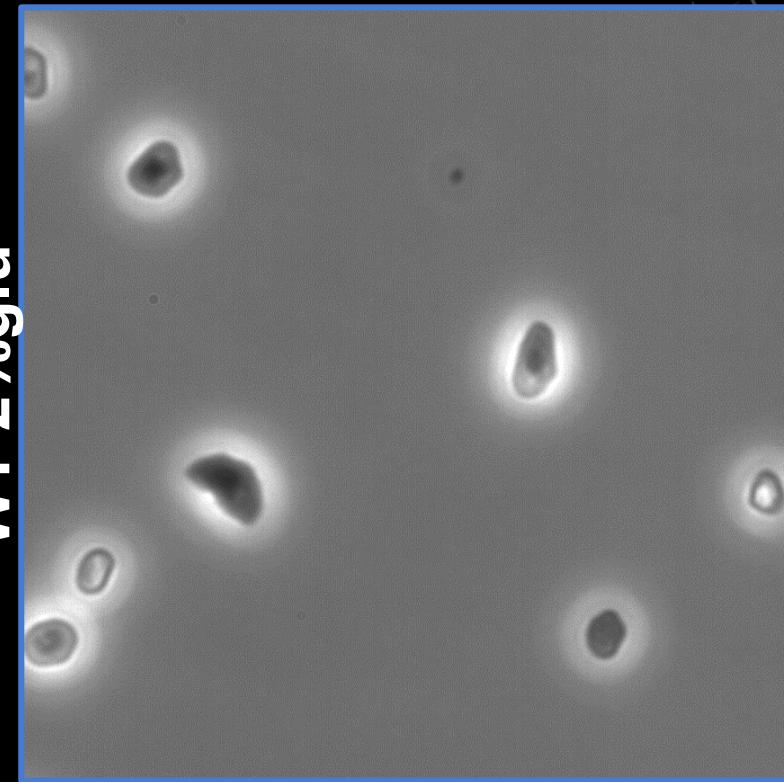
2% glu
22.3

DIRECTLY MEASURE CELL SIZE USING MICROSCOPES

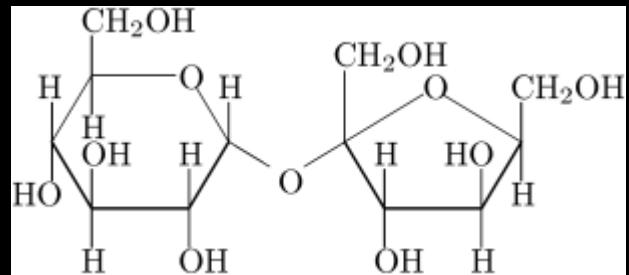
dTrmB 2%glu



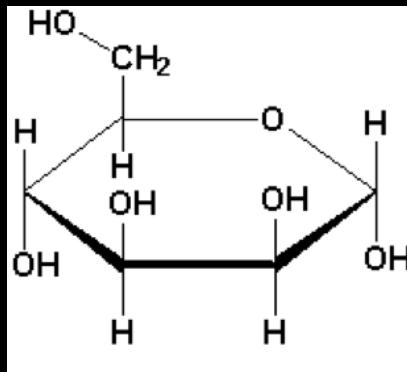
WT 2%glu



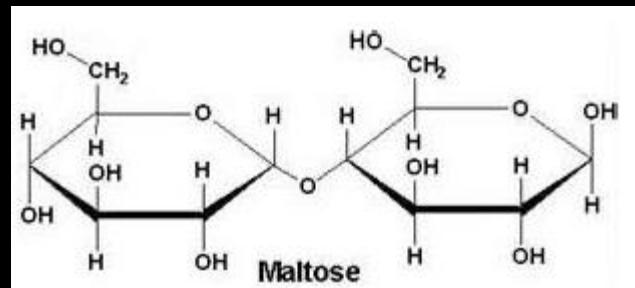
ALTERNATIVE SUGARS:



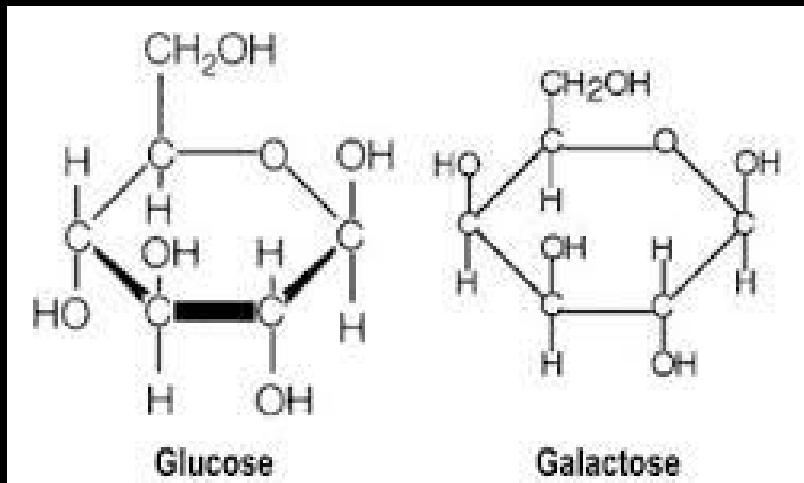
Sucrose



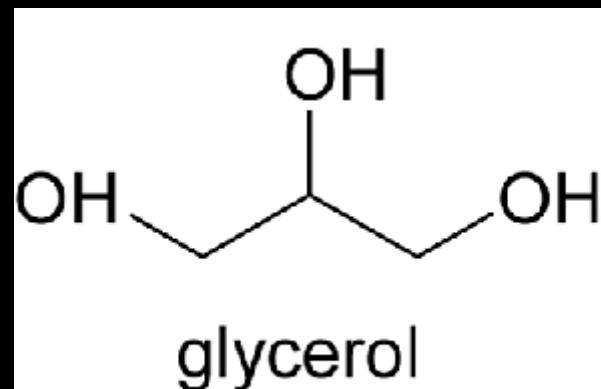
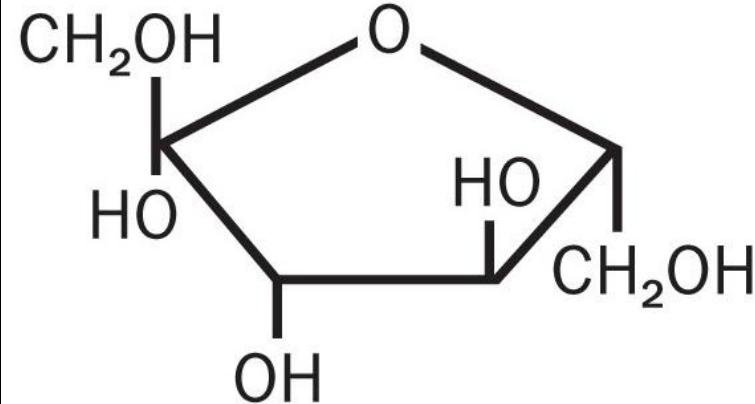
Mannose



Maltose



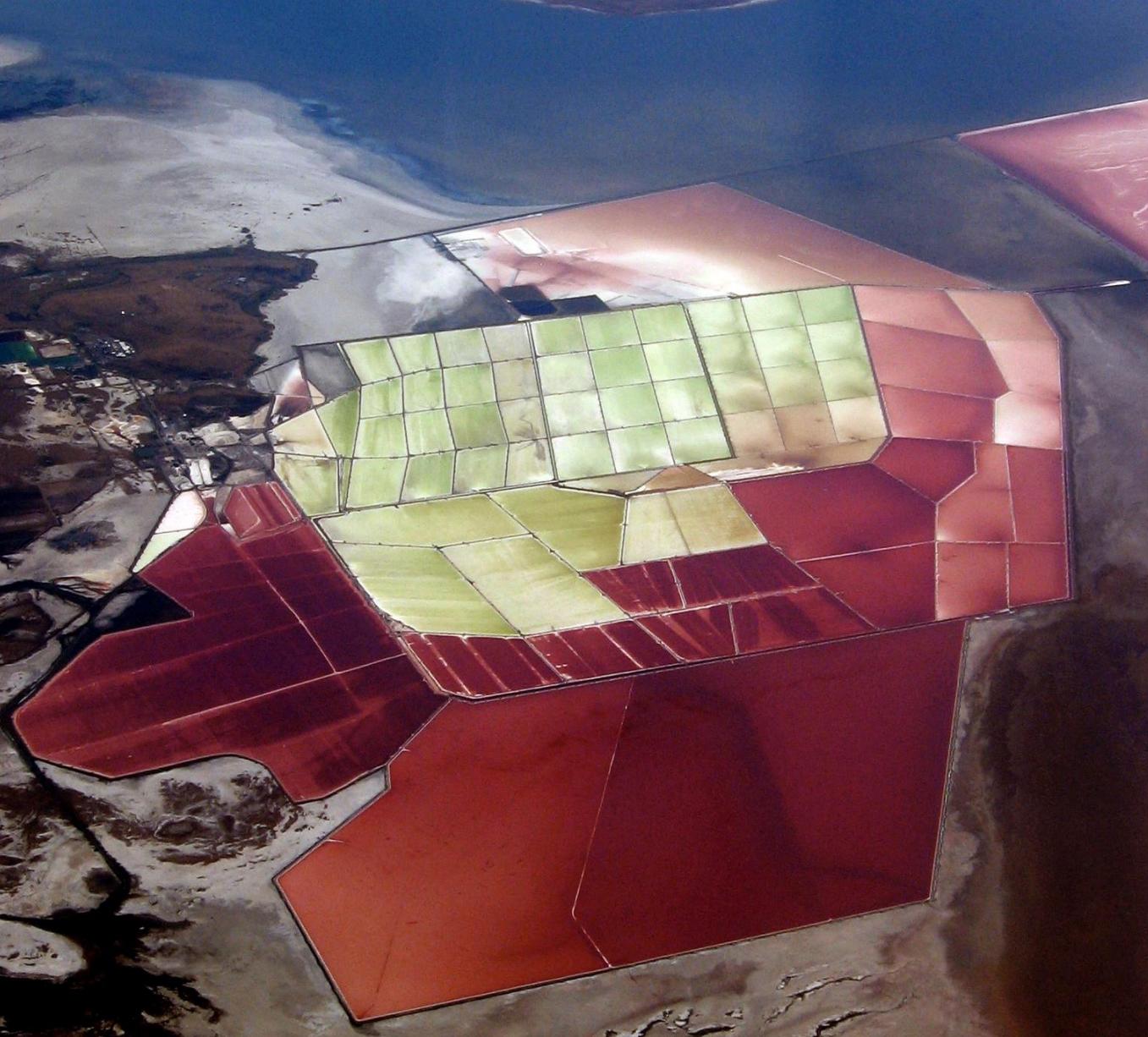
Fructose



glycerol

EXPERIMENTAL OUTLINE FOR NEXT TIME

1. Grow parent and $\Delta trmB$ *Hca. Hispanica* strains
2. Take OD of initial cultures and design plate layout for OD measurements
3. Calculate dilution necessary for the growth curve measurements
4. Take microscopy images of the parent and $\Delta trmB$ strains for later quantification.



Questions?