Microorganisms in Diverse Environments

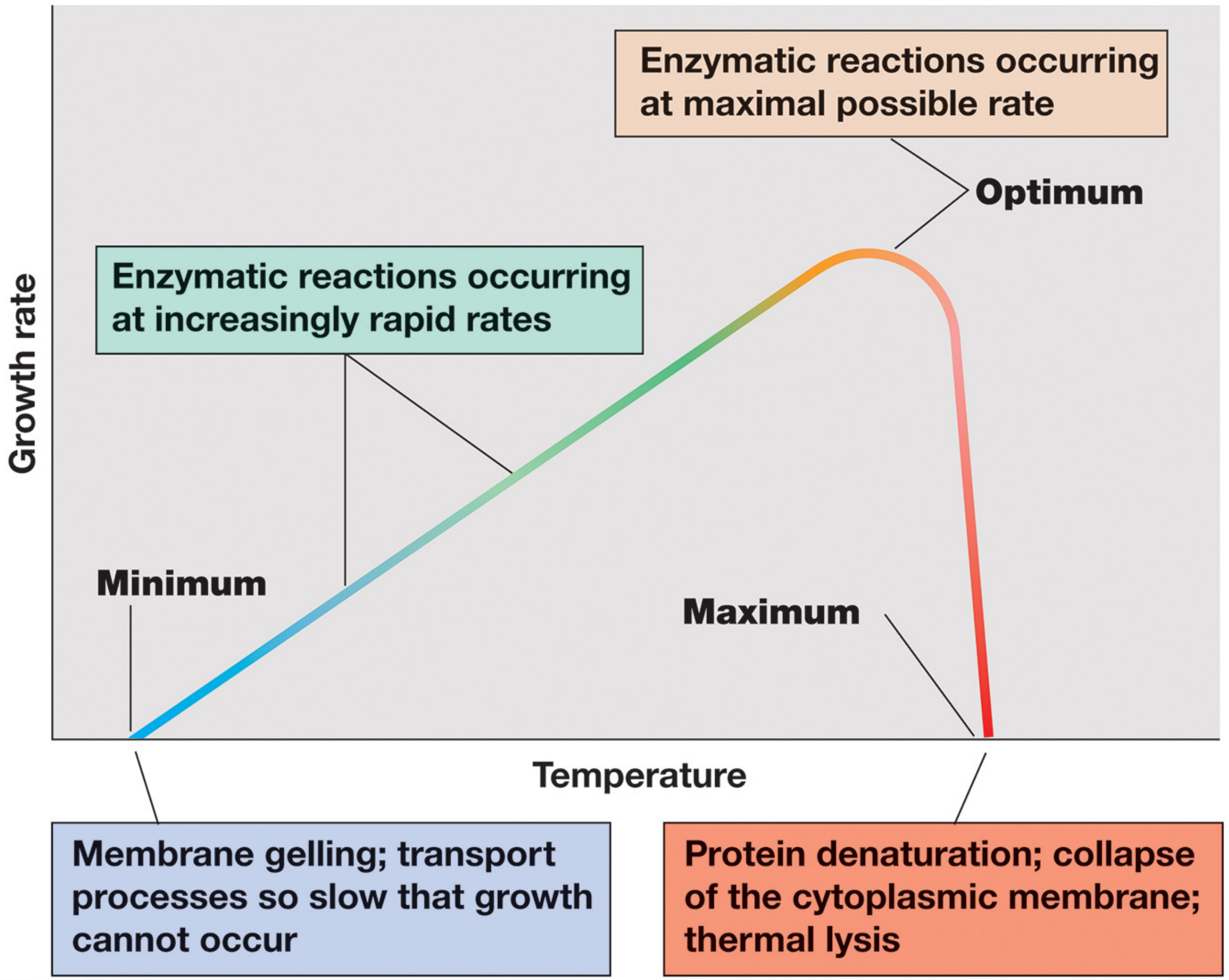
Diversity of growth conditions for microorganism:

- Temperature
- O_2 availability
- Light availability
- pH
- Water availability
- Nutrient availability
- Toxic contaminants

$\Bigg\}$ Gradients and extremes

Temperature Classes of Microorganisms

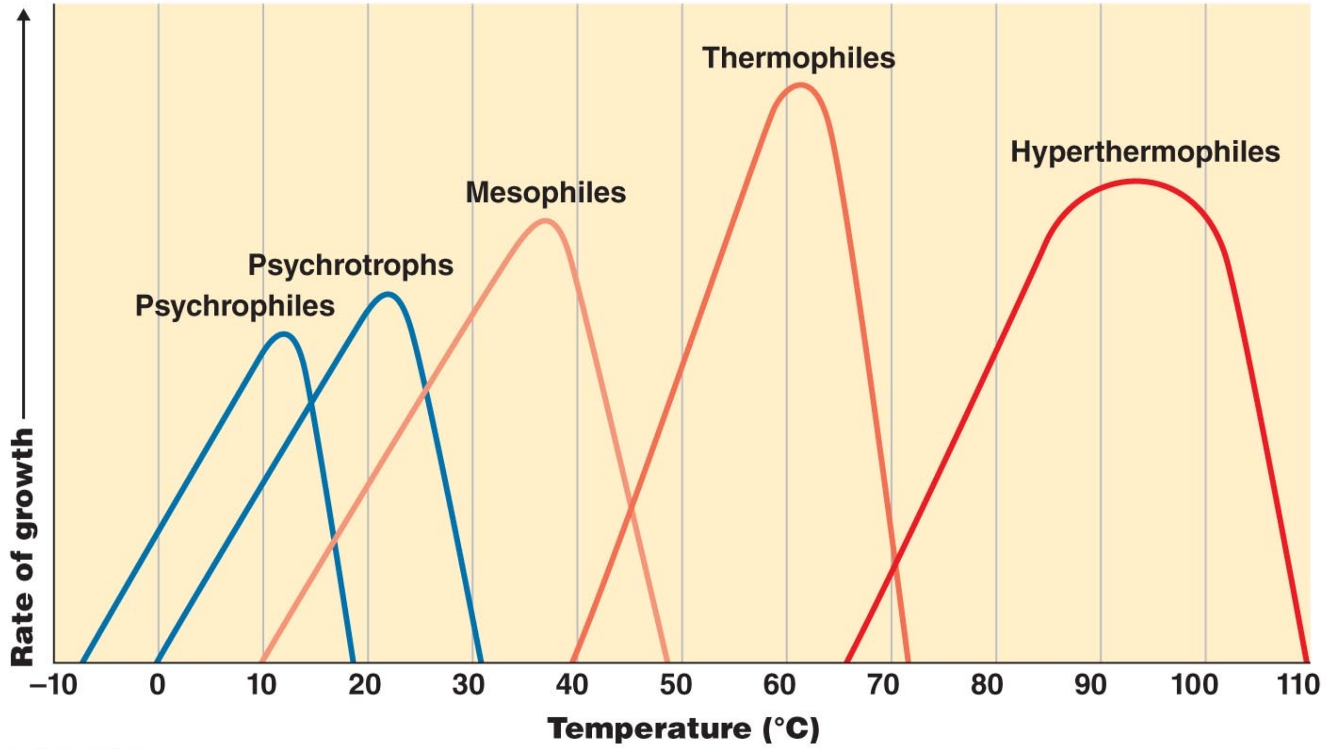
- Cardinal Temperatures
  - **Minimum**, **optimum**, and **maximum** temperatures at which an organism grows
  - Characteristic of any given microorganism
  - Differ dramatically between species
  - Range is typically < 40°C
  - At optimum, all/most cellular components are functioning at maximum rate



Temperature Classes of Microorganisms

Broad classes related to growth temperature optima

- **Psychrophile:** low, found in cold environments
- **Psychrotroph:** low-mid can grow at cold temperatures
- **Mesophile:** midrange, most commonly studied
- **Thermophile:** high, found in hot environments
- **Hyperthermophile:** very high, found in extremely hot habitats such as hot springs and deep-sea hydrothermal vents



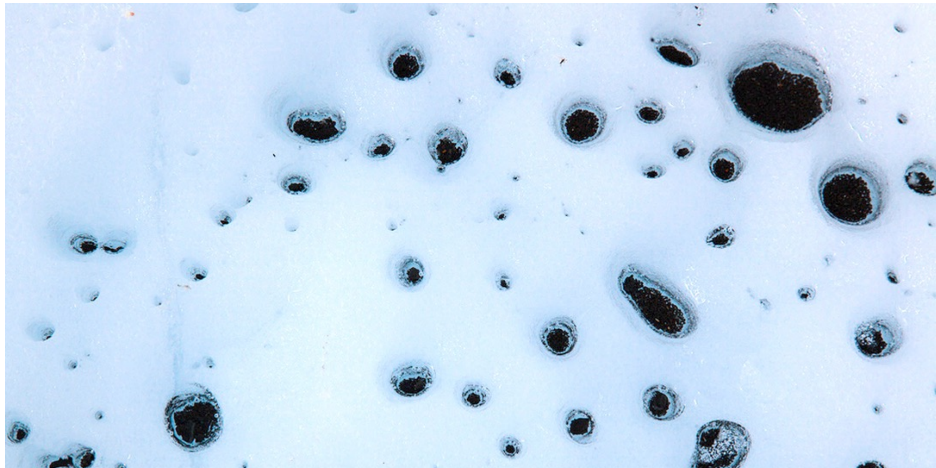
Extremophiles

Extremophiles

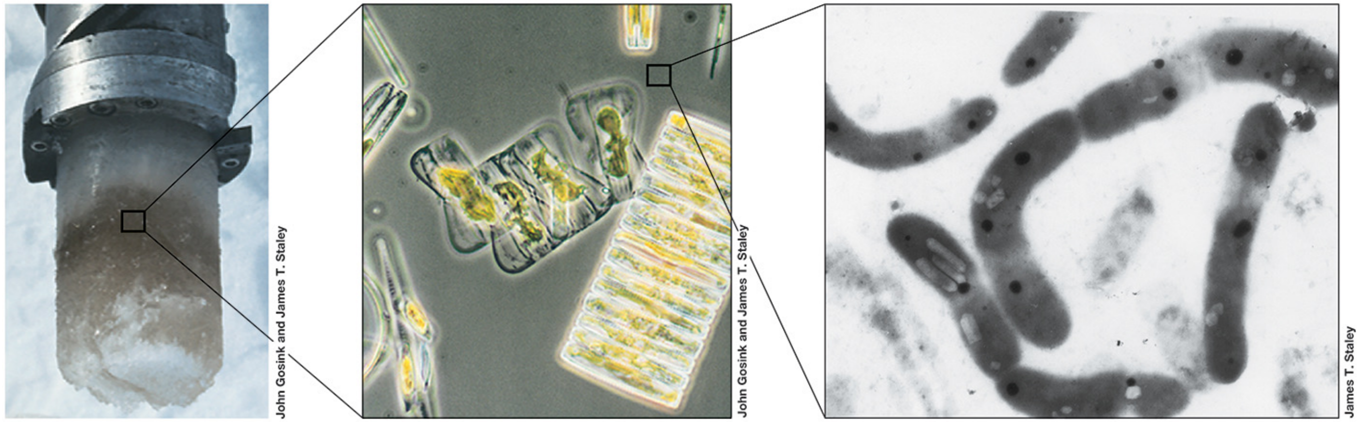
Microbial Life in the Cold

Cold Environments:

- Much of Earth's surface is cold
  - Oceans - 5°C
  - Arctic and Antarctic are permanently frozen or rarely unfrozen
- **Constantly** v s **seasonally** cold



Antarctic Microbial Habitats and Microorganisms



(a) (b) (c)

(d) (e)

(a) A core of frozen seawater from McMurdo Sound, Antarctica. Coloration due to pigmented microorganisms.
(b) Phototrophic microorganisms within the core, most are diatoms or green algae (both euk. phototrophs).
(c) Transmission electron micrograph of *Polaromonas*, a bacterium that lives in sea ice with optimal growth at 4°C.

(d) Permanently ice-covered lake Bonney, Antarctica. The water column under the ice contains a diverse array of *Bacteria, Archaea*, and microbial eukaryotes.
(e) Garwood Glacier, Antarctica. Glaciers and subglacial lakes are full with of microbial life.

Microbial Life in the Cold

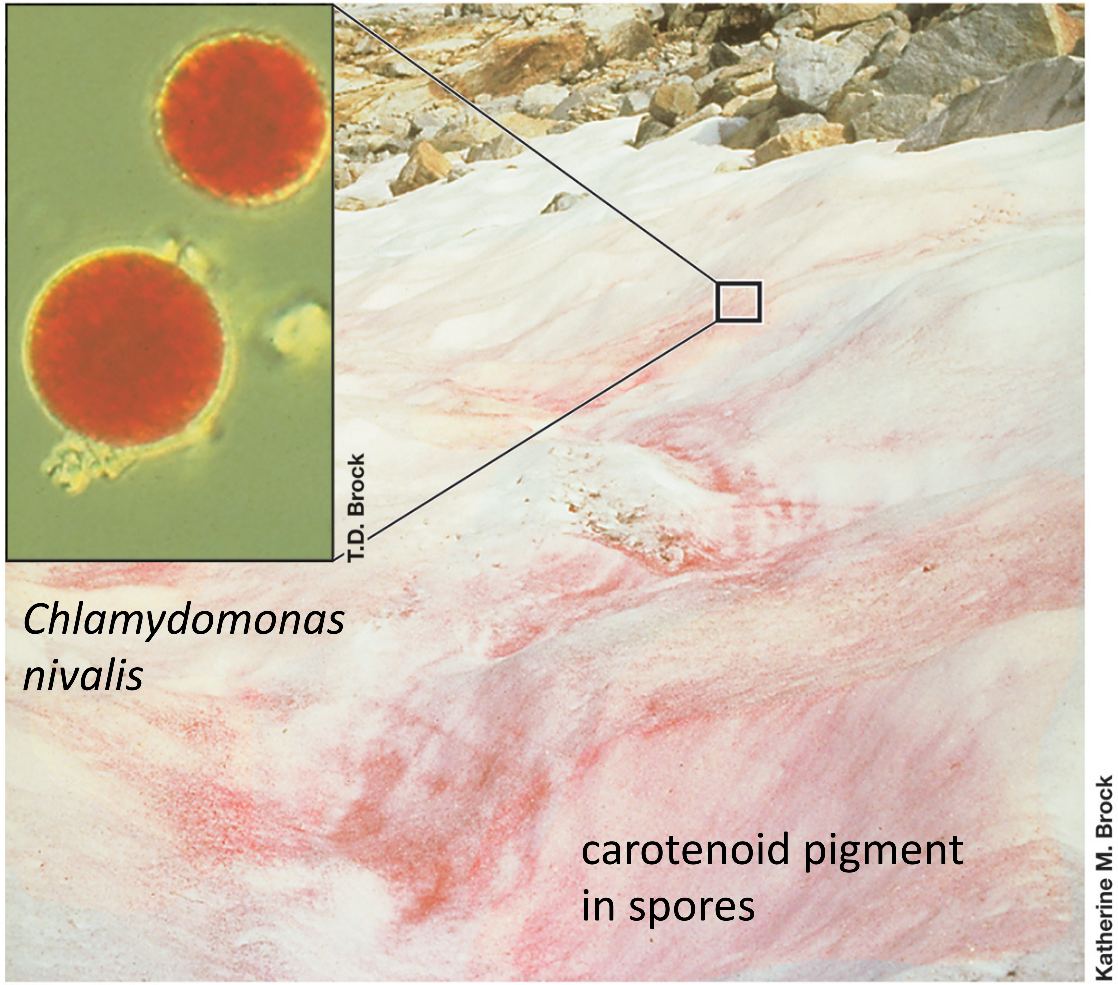
- Psychrophilic and Psychrotolerant Microorganisms
  - **Psychrophiles:** optimal growth temperature ≤ 15°C, maximum < 20°C, minimum ≤ 0°C
    - Constantly cold environments
    - Found in polar regions, permanent snowfields, glaciers (Figure 4.23)
  - **Psychrotolerant:** can grow at 0°C but have optima of 20°C to 40°C
    - More widely distributed in nature than psychrophiles
    - Isolated from soils and water in temperate climates and food at 4°C

Microbial Life in the Cold

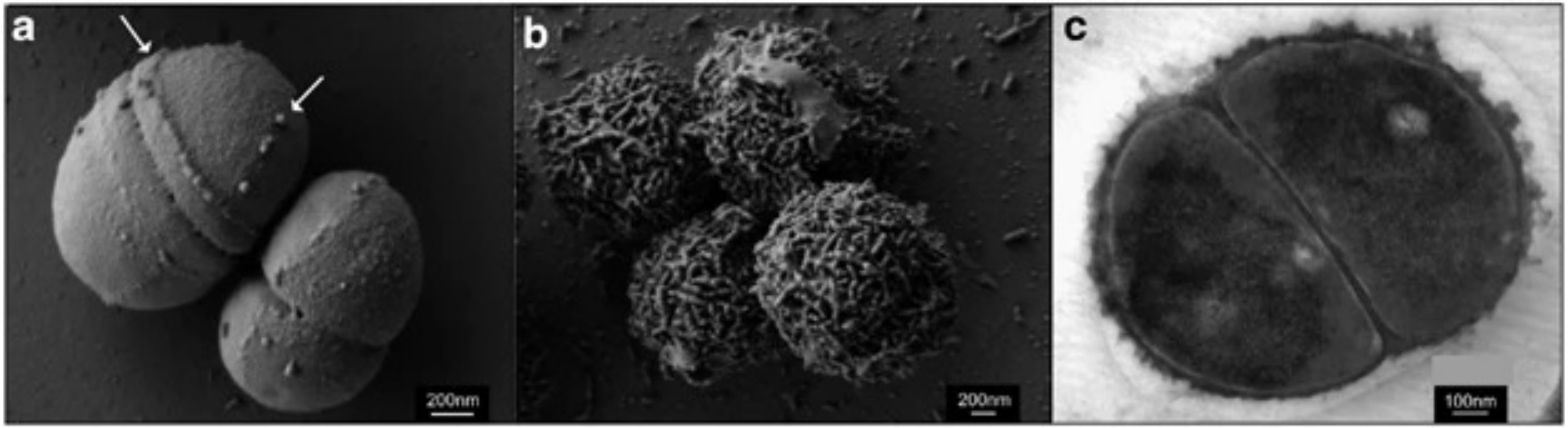
Psychrophilic algae and bacteria often grow within and under sea ice in polar regions.

They can also be found on the surfaces of permanent snowfields and glaciers.

- This alga grows within the snow as a green-pigmented vegetative cell and then sporulates.
- As the snow melts the spores become concentrated on the surface.



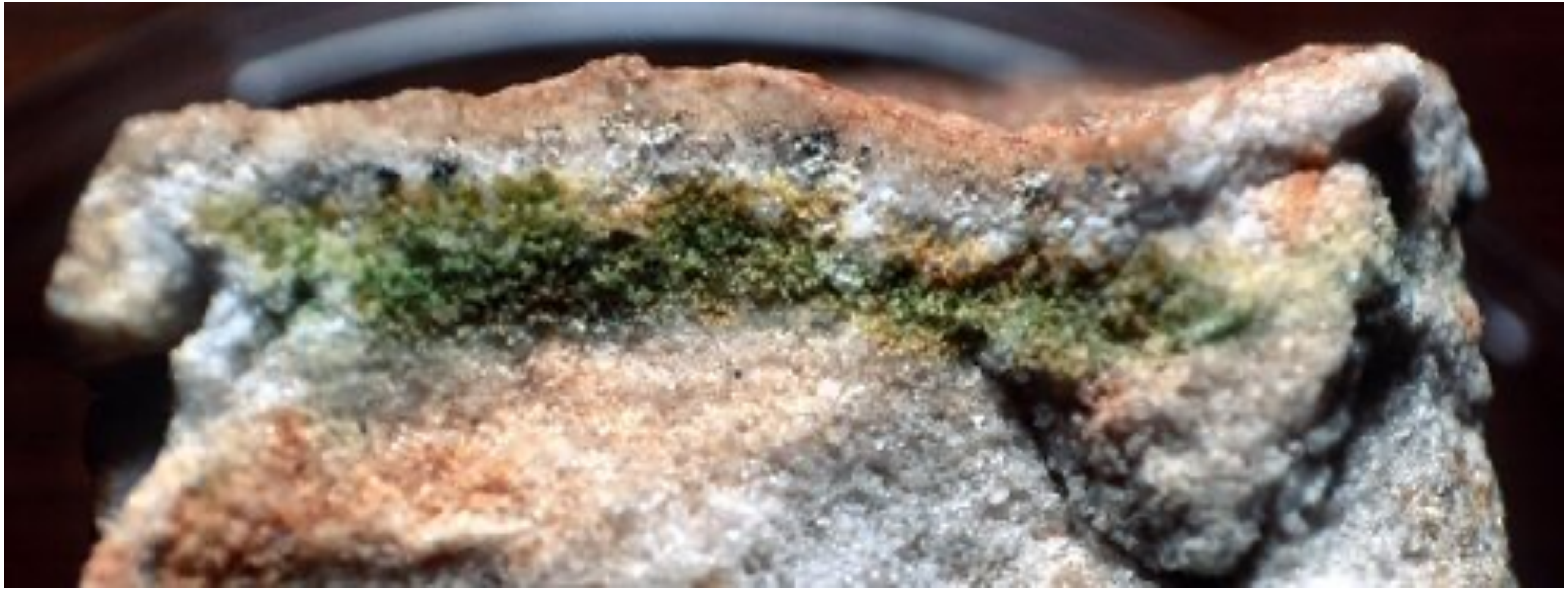
Microbial Life in the Cold



Planococcus halocryophilus

Isolated from high Arctic permafrost, grows and divides at −15 °C and is metabolically active at −25 °C.

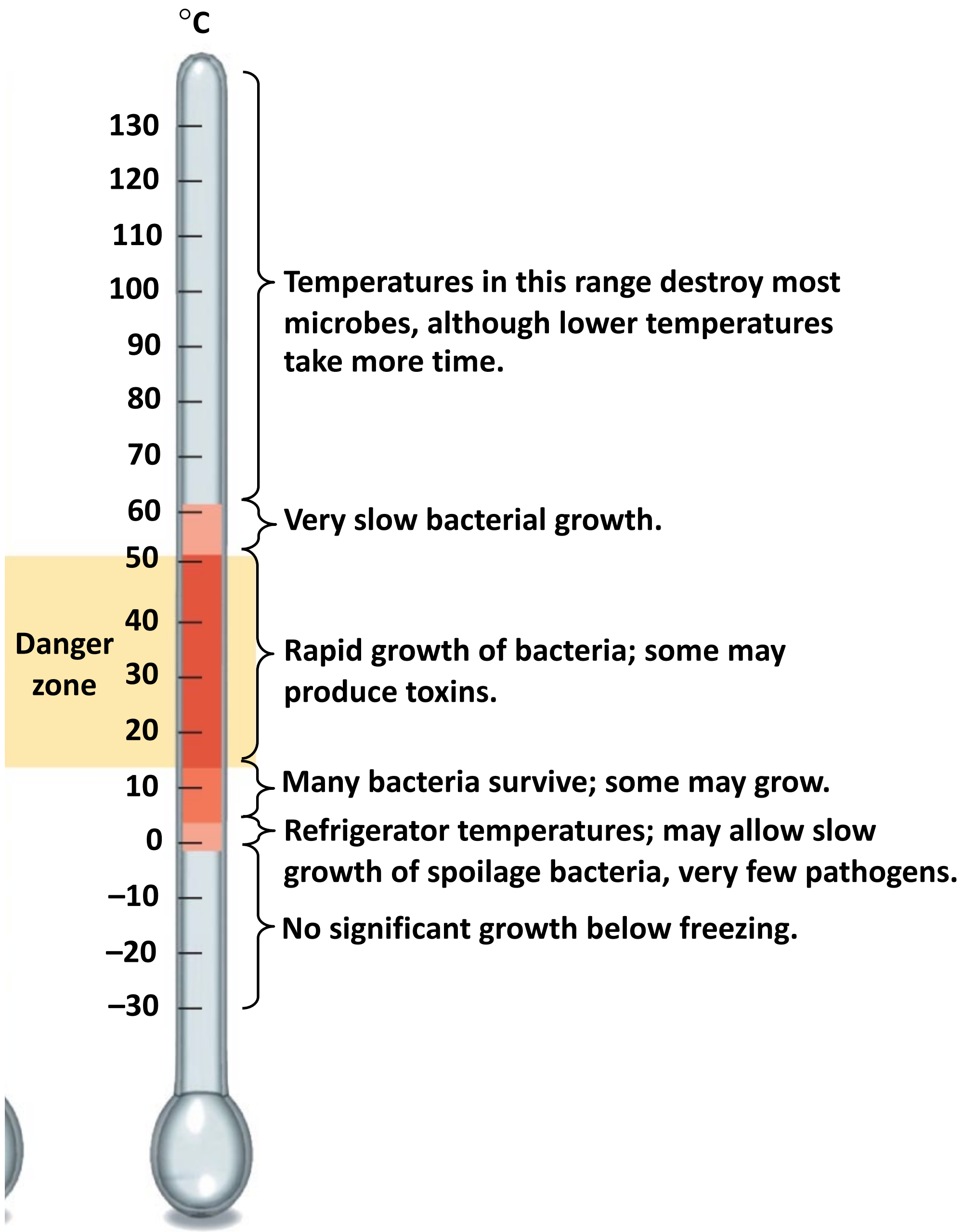
Microbial Life in the Cold

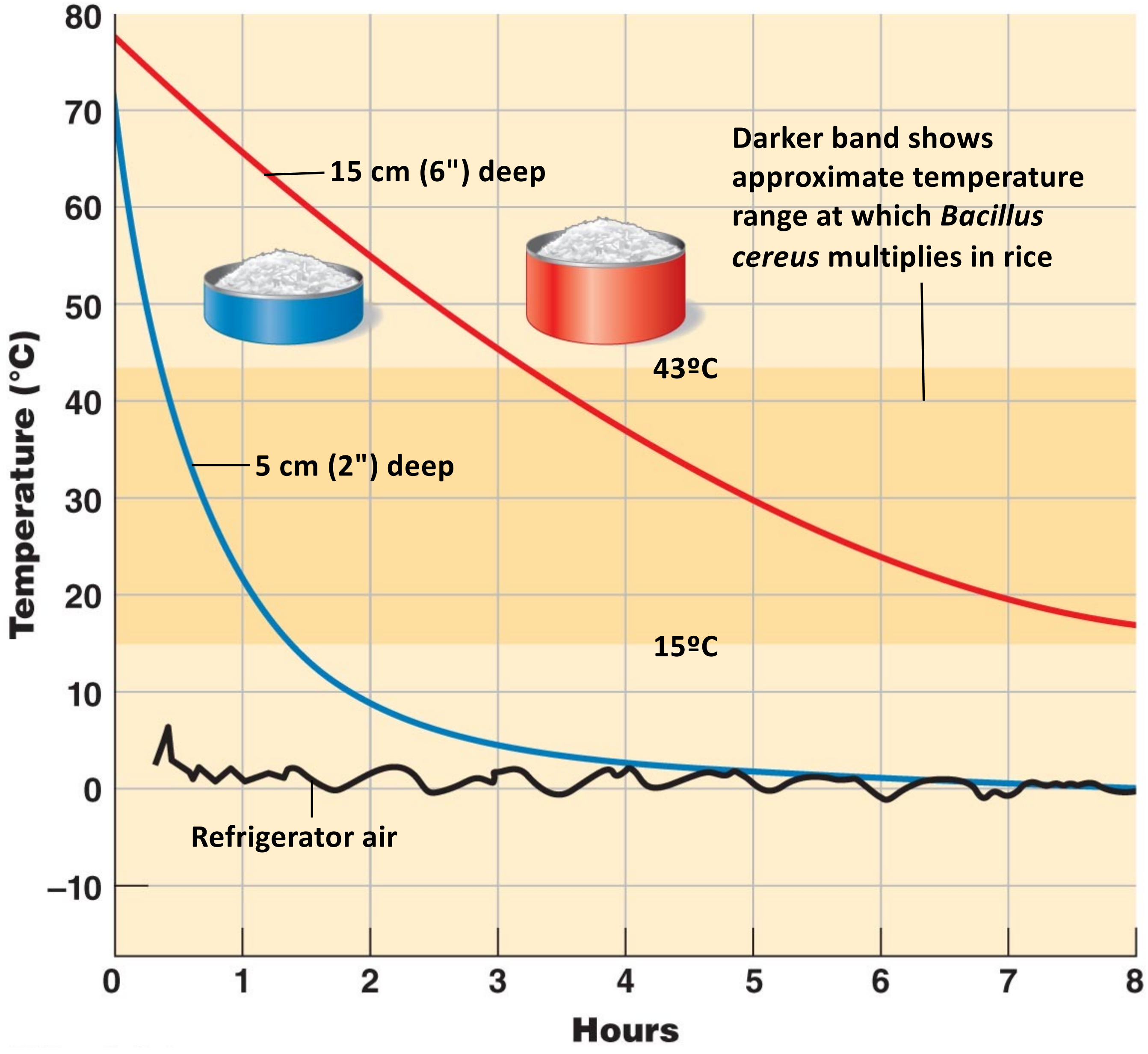


Cryptoendolith

Psychrotolerant microorganisms are more widely distributed in nature than are psychrophiles

Food preservation temperatures.

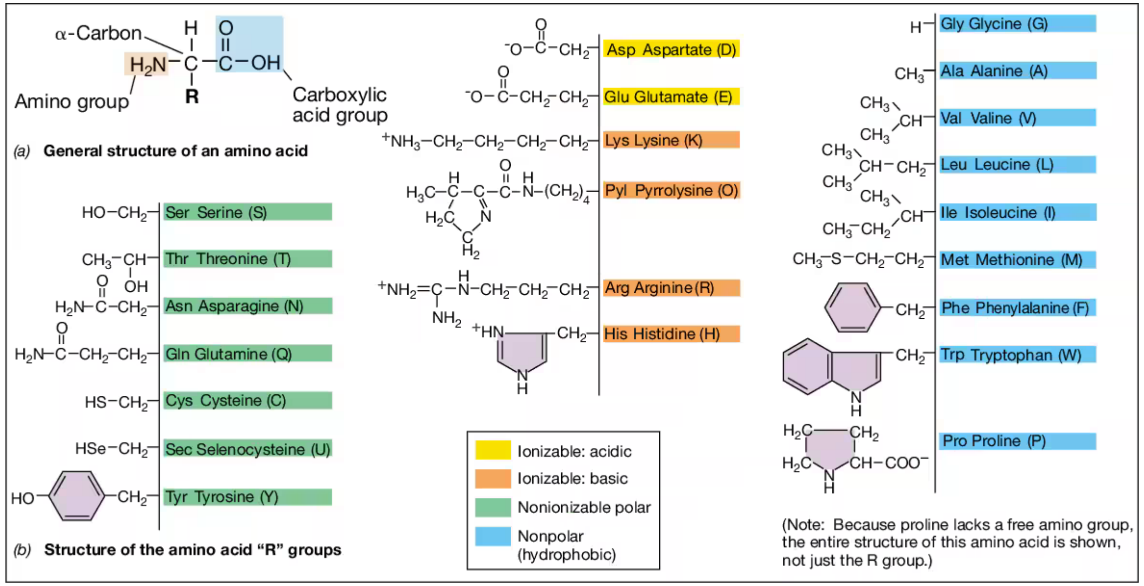


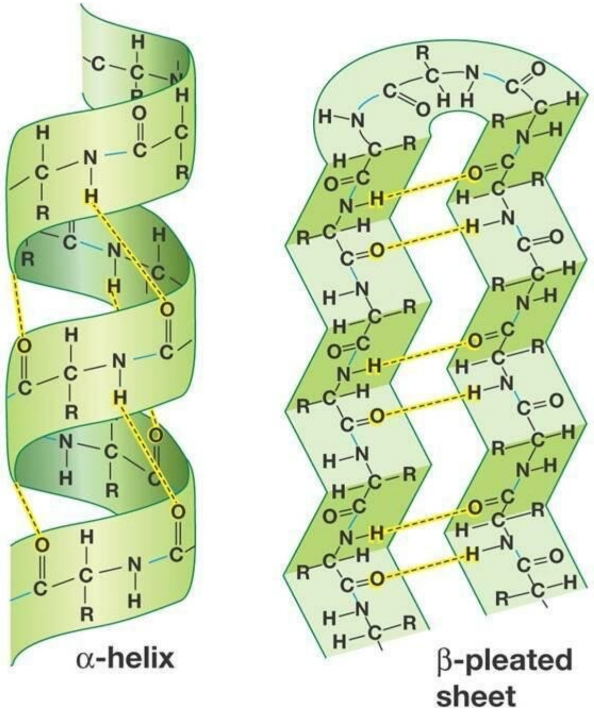


80
70
60
50
40
30
20
10
0
–10
Temperature (°C)
0
1
2
3
4
5
6
7
8
Hours
15 cm (6") deep
5 cm (2") deep
Refrigerator air
43ºC
15ºC
Darker band shows approximate temperature range at which *Bacillus cereus* multiplies in rice

Molecular Adaptations to Life in the Cold

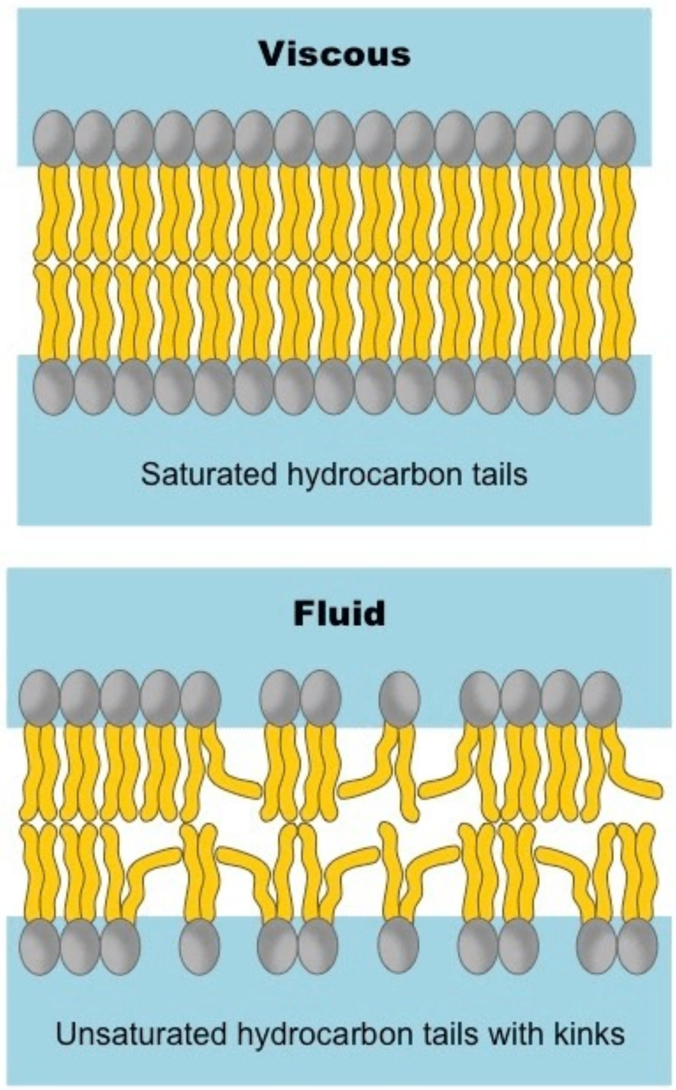
- Production of enzymes that function optimally in the cold
  - more α-helices than β-sheets → greater flexibility for catalysis at cold temperatures
  - more polar and fewer hydrophobic amino acids





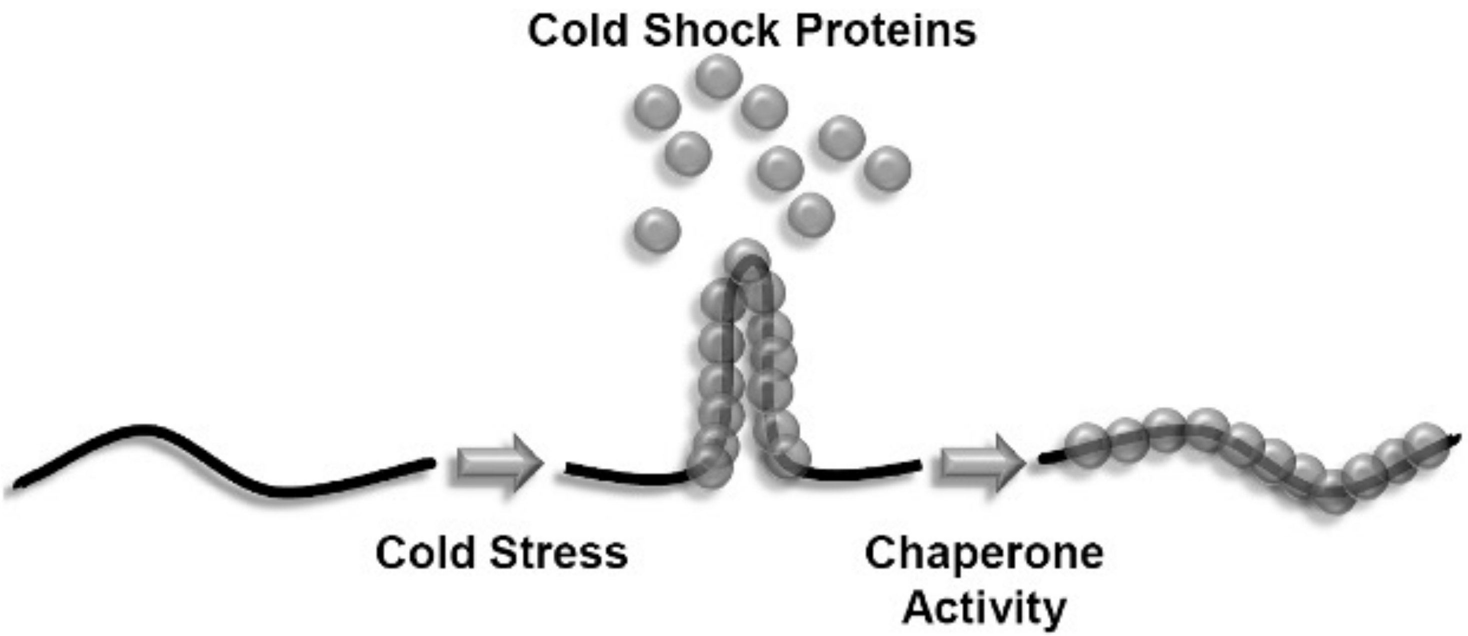
Molecular Adaptations to Life in the Cold

- Cytoplasmic membranes function at low temperatures.
  - higher unsaturated and shorter-chain fatty acid content
  - some polyunsaturated fatty acids, which remain flexible at very low temperatures



Molecular Adaptations to Life in the Cold

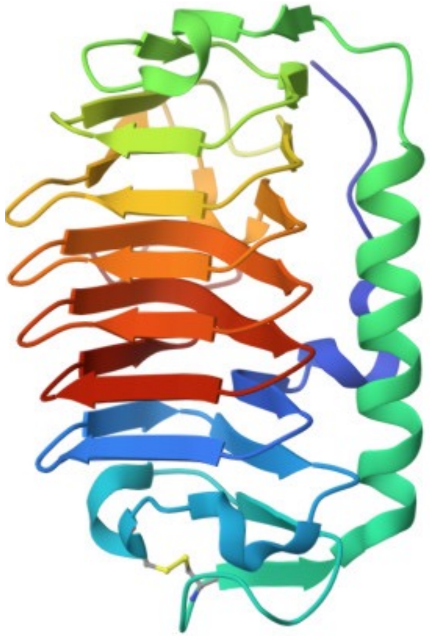
- Cold shock proteins (chaperones)



Molecular Adaptations to Life in the Cold

- Cryoprotectants - prevent formation of ice crystals
  - e.g., antifreeze proteins, certain solutes (glycerol, sugars, ethanol)

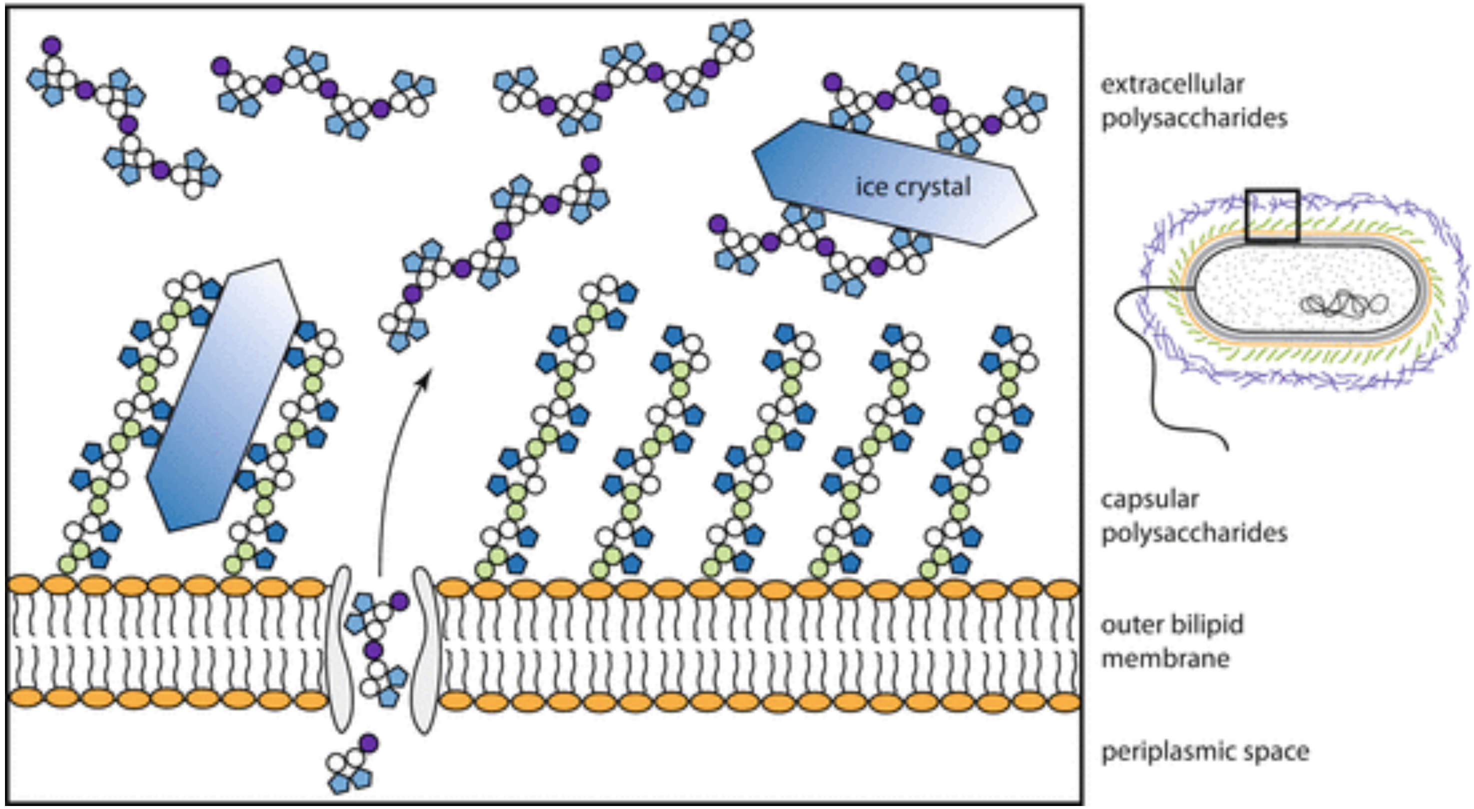
- Exopolysaccharide cell surface slime
  - extracellular carbohydrate polymers



Antarctic sea ice bacterium *Colwellia*

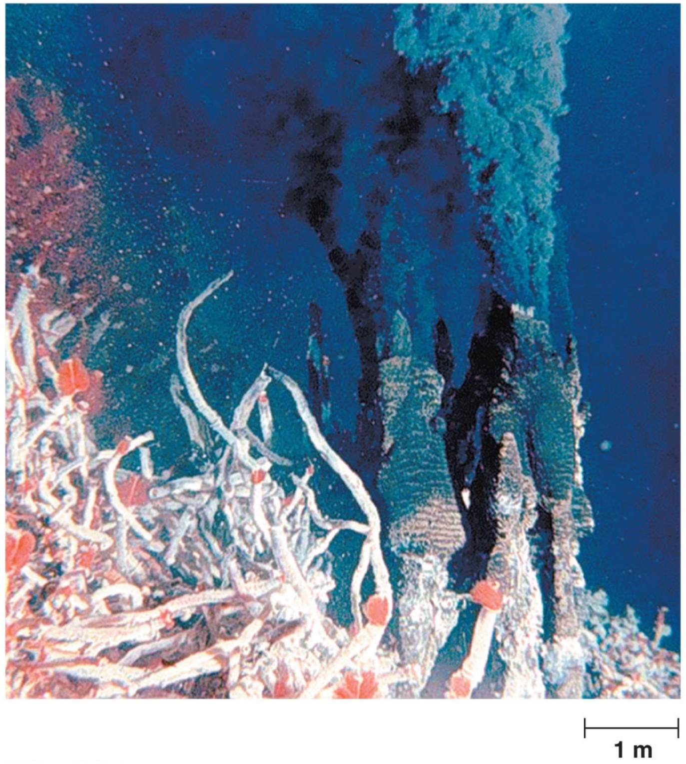
Molecular Adaptations to Life in the Cold

- Exopolysaccharide cell surface slime
  - extracellular carbohydrate polymers



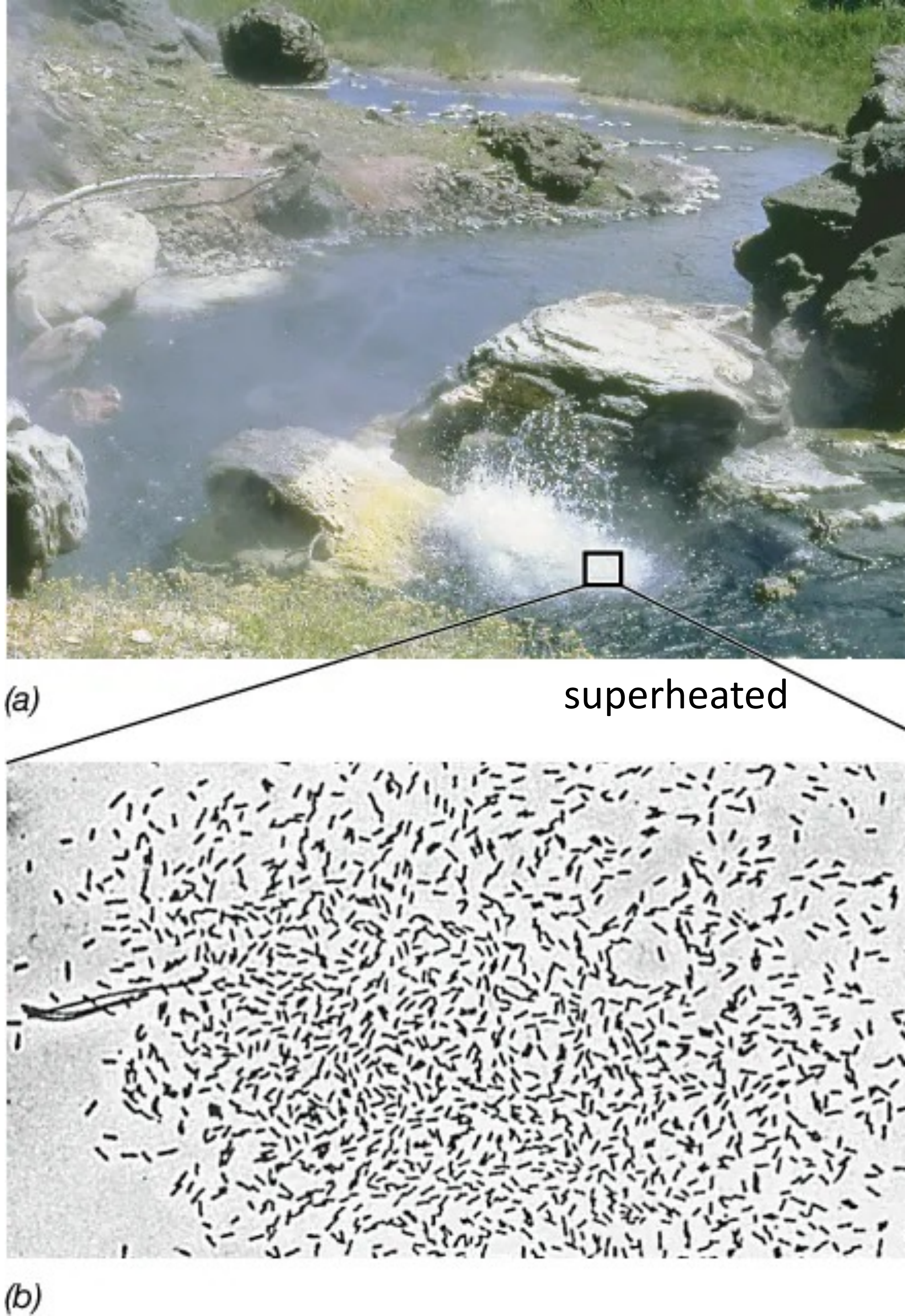
Microbial Life at High Temperatures

- Thermal Environments
  - **Thermophiles:** growth temperature optima between 45°C and 80°C
  - **Hyperthermophiles:** optima greater than 80°C
    - inhabit hot environments, including boiling hot springs and seafloor hydrothermal vents, that can experience temperatures in excess of 100°C

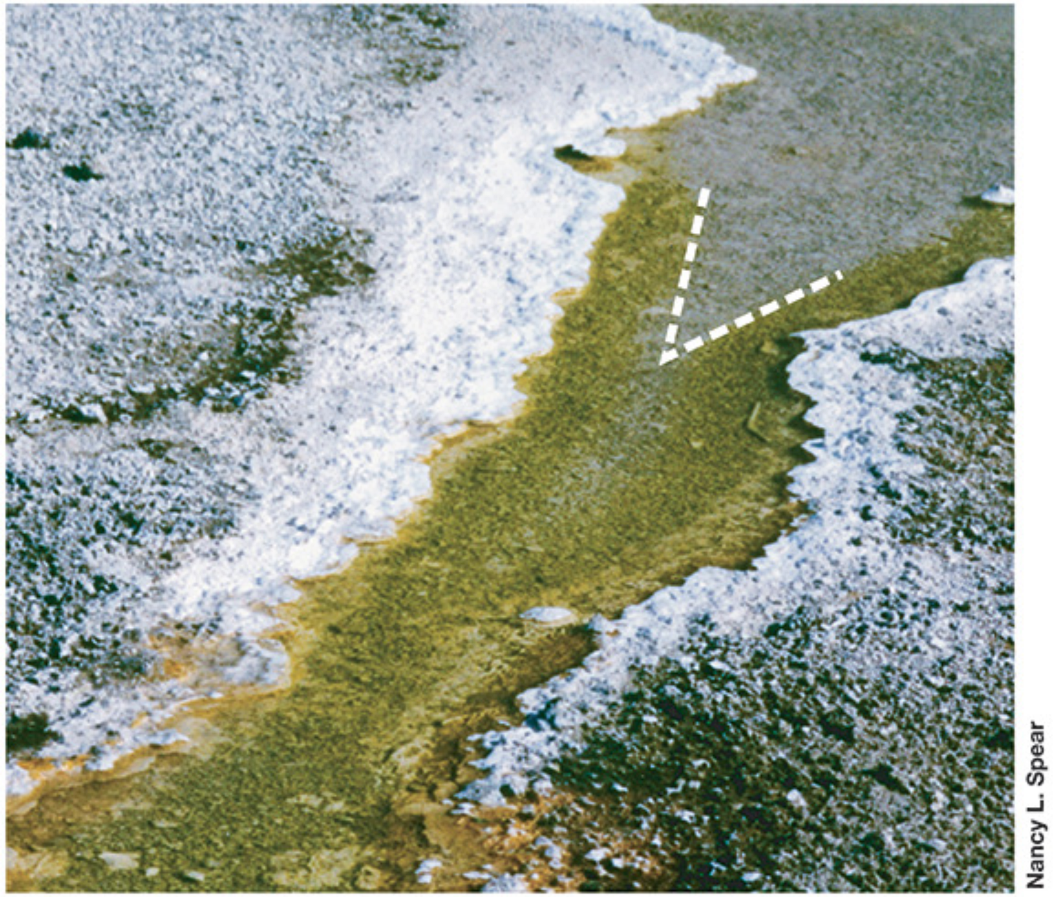


Microbial Life at High Temperatures

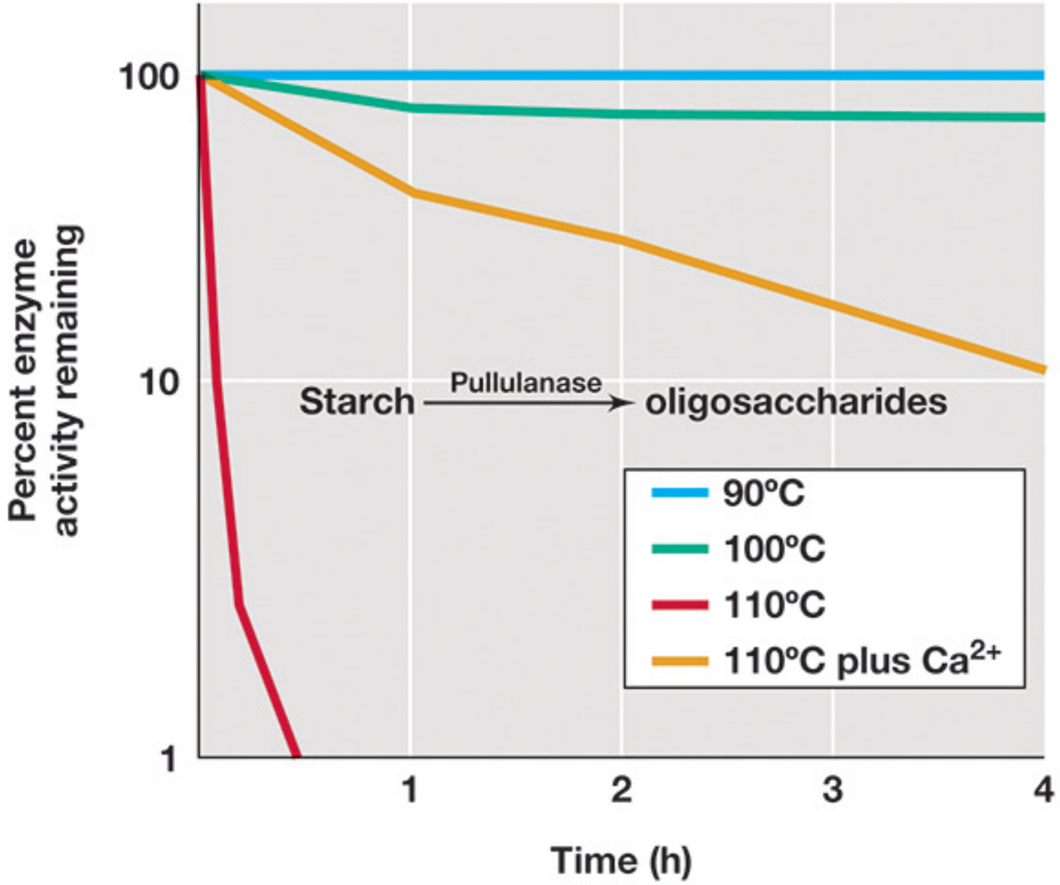
- Hyperthermophiles and Thermophiles
  - Thermophiles - optima 45–80°C
    - moderately or intermittently hot environments
  - Hyperthermophiles
    - inhabit boiling hot springs
    - chemoorganotrophic and chemolithotrophic species present
    - Fast generation times (can be 1h)
    - high prokaryotic diversity (both **Archaea** and **Bacteria** represented)



Hot Spring Microbes and Their Heat-Stable Enzymes



(a)



(b)

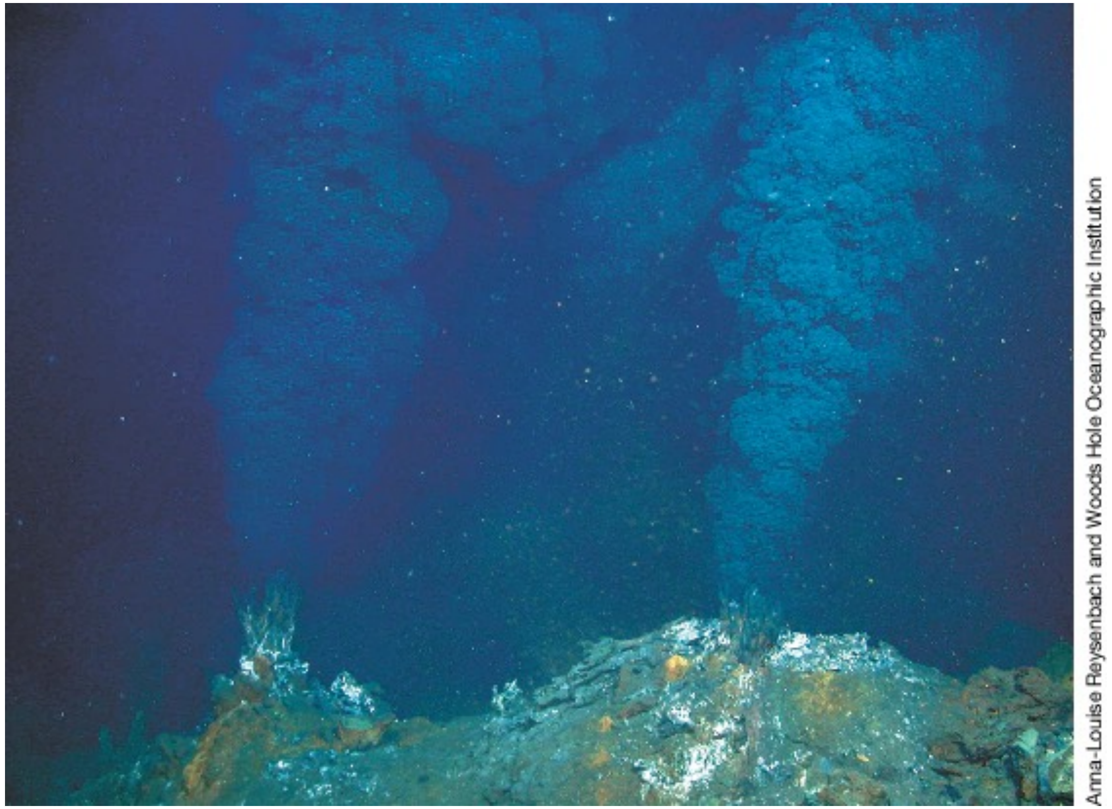
Presently Known Upper Temperature Limits for Growth of Living Organisms

Group	Upper temperature limits (°C)
Macroorganisms	
Animals	
Fish and other aquatic vertebrates	38
Insects	45–50
Ostracods (crustaceans)	49–50
Plants	
Vascular plants	45 (60 for one species)
Mosses	50
Microorganisms	
Eukaryotic microorganisms	
Protozoa	56
Algae	55–60
Fungi	60–62
Bacteria and Archaea	
Bacteria	
Cyanobacteria	73
Anoxygenic phototrophs	70–73
Chemoorganotrophs/chemolithotrophs	95
Archaea	
Chemoorganotrophs/chemolithotrophs	122

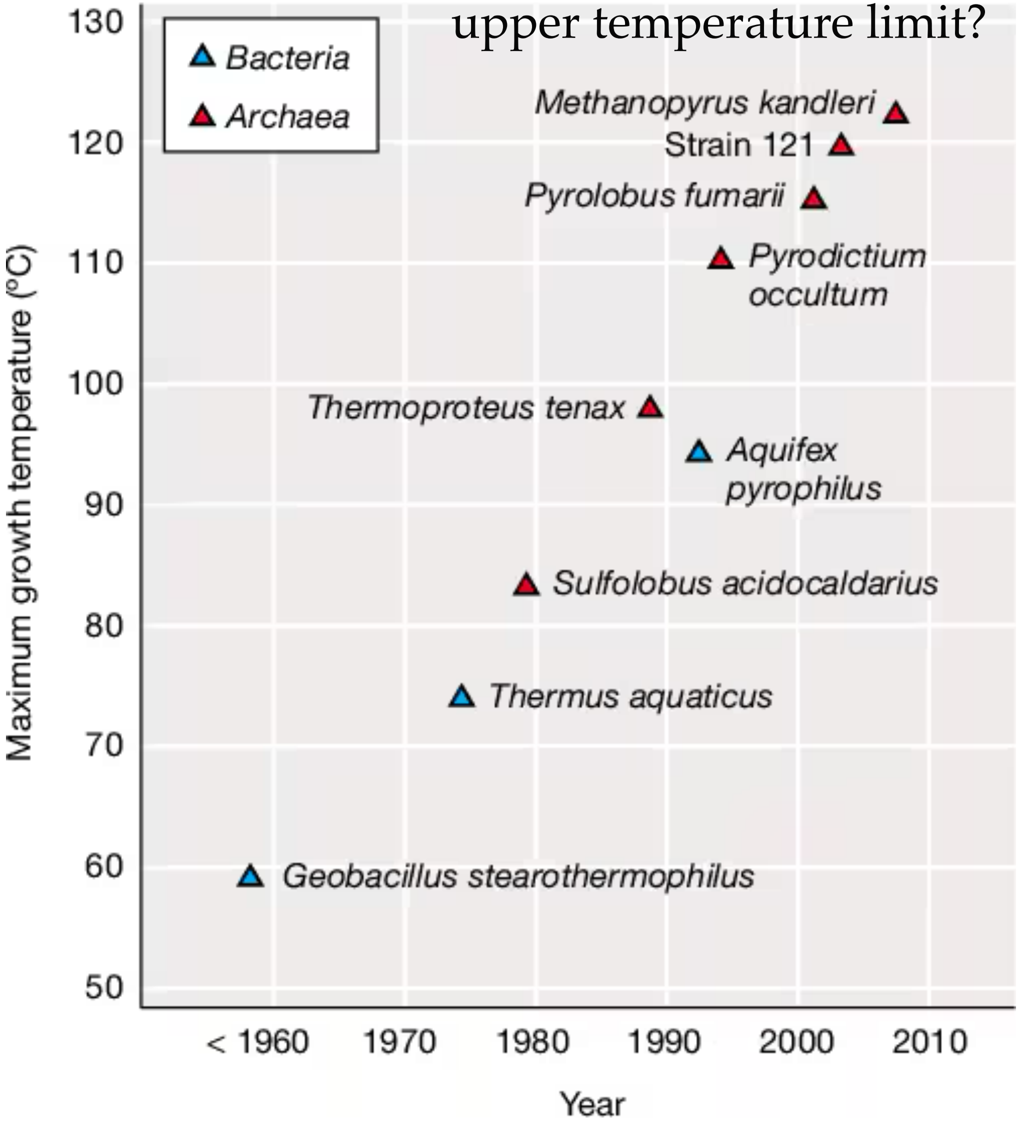
Above 65°C, only prokaryotic life forms thrive

extensive diversity

Presently Known Upper Temperature Limits for Growth of Living Organisms



Back smokers emit hydrothermal vent fluid at 250–350°C or higher



Methanopyrus: most thermophilic, up to 122°C

Microbial Life at High Temperatures

Protein and Membrane Stability at High Temperatures

- Enzymes and proteins more heat-stable and function optimally at high temperatures
  - AA changes compared to mesophiles
  - Increased ionic bonding and highly hydrophobic interiors

- Thermophilic and hyperthermophilic enzymes commercially useful
  - Prolong shelf life (stability)
  - **e.g., Taq polymerase** for polymerase chain reaction [PCR]

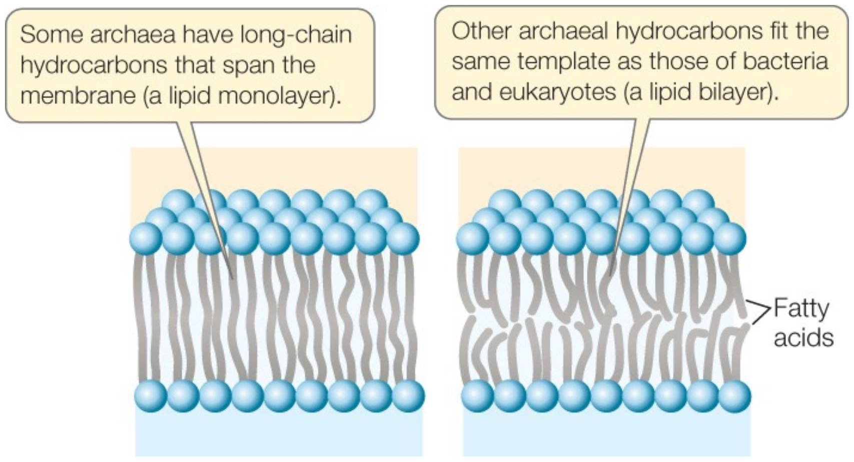
Microbial Life at High Temperatures

Protein and Membrane Stability at High Temperatures

- Heat naturally works to peel apart the lipid bilayer
- Cytoplasmic membranes must be heat stable

  - **Bacteria** have lipids rich in long-chain and saturated fatty acids, fewer unsaturated fatty acids.

  - Most hyperthermophiles (**Archaea**) have C_{40} hydrocarbons made of repeating isoprene units
  - membrane forms lipid monolayer rather than bilayer.



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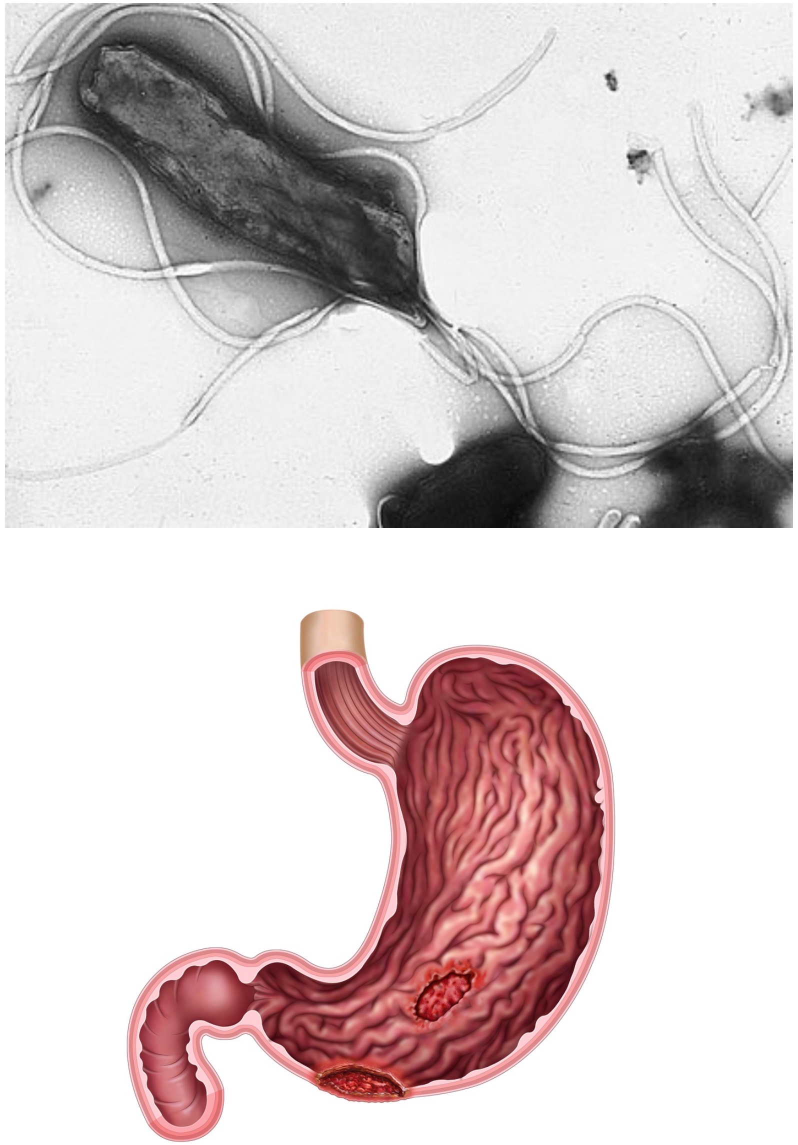
Oxygen and Microbial Growth

Microorganisms can be grouped according to their relationship with O_2

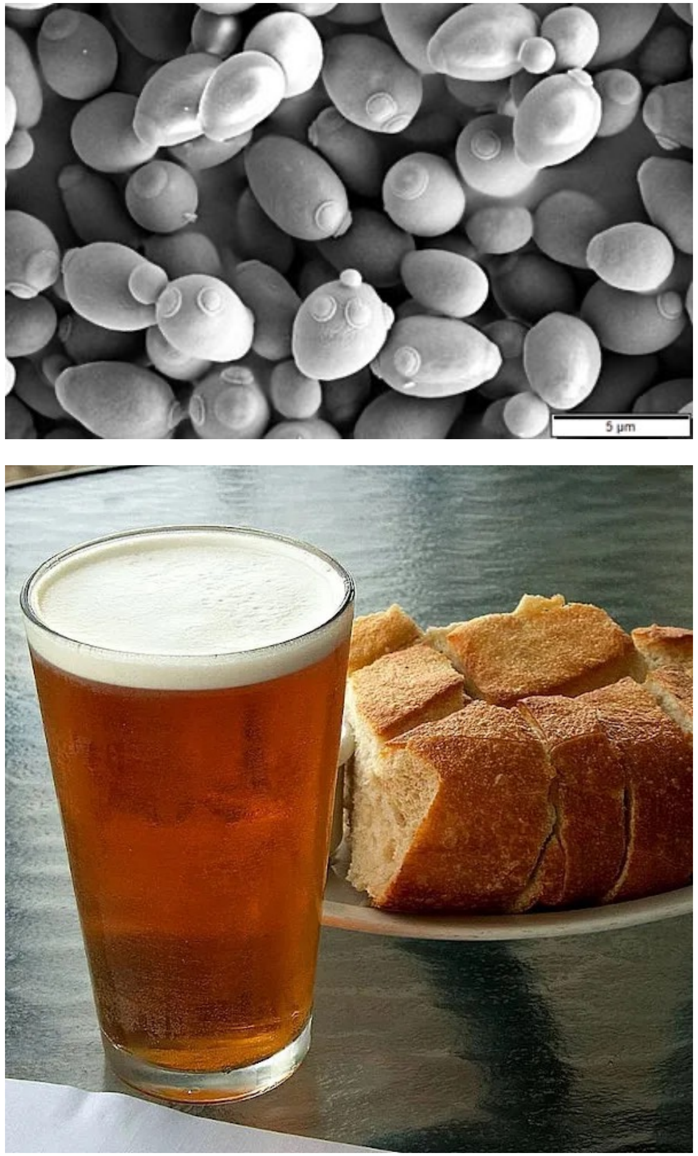
Oxygen Classes of Microorganisms:

- **Aerobes:** grow at full O_2 tension (~21%) and respire O_2
- **Microaerophiles:** can use O_2 only at levels reduced from that in air (microxic) due to limited respiration or oxygen sensitivity
- **Facultative** organisms: can live with or without oxygen

Mycobacterium tuberculosis



Helicobacter pylori



Saccharomyces cerevisiae

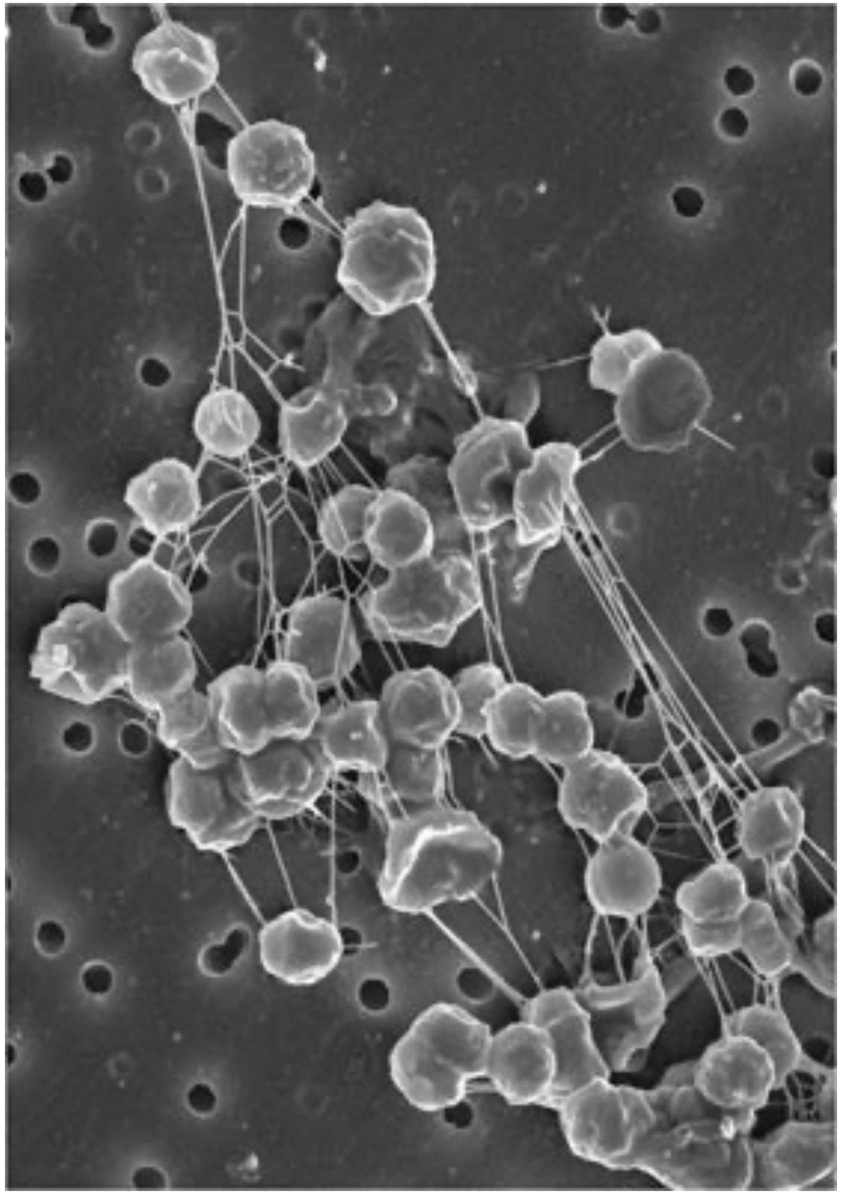
Oxygen and Microbial Growth

Microorganisms can be grouped according to their relationship with O_2

Oxygen Classes of Microorganisms:

- **Anaerobes:** cannot respire oxygen
  - **Aerotolerant anaerobes:** tolerate oxygen and grow in its presence even though they cannot respire
  - Obligate anaerobes: inhibited or killed by oxygen, (**e.g., Bacteria** and **Archaea**, few fungi, and few protozoa)

Anoxic (oxygen free) habitats: mud, bogs, marshes, animal intestines, other diverse habitats



Oxygen Relationships of Microorganisms

Aerobes

Group	Relationship to O_2	Type of metabolism	Example[a]	Habitat[b]
Obligate	Required	Aerobic respiration	**Micrococcus luteus** (B)	Skin, dust
Facultative	Not required, but growth better with O_2	Aerobic respiration, anaerobic respiration, fermentation	**Escherichia coli** (B)	Mammalian large intestine
Microaerophilic	Required but at levels lower than atmospheric	Aerobic respiration	**Spirillum volutans** (B)	Lake water

Anaerobes

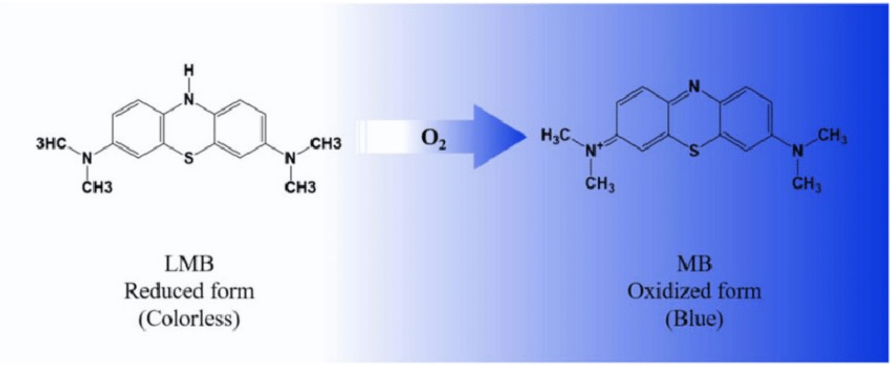
Group	Relationship to O_2	Type of metabolism	Example[a]	Habitat[b]
Aerotolerant	Not required, and growth no better when O_2 present	Fermentation	**Streptococcus mutans** (B)	Oral cavity
Obligate	Harmful or lethal	Fermentation or anaerobic respiration	**Methanobacterium formicicum** (A)	Sewage sludge, anoxic lake sediments

[a]Letters in parentheses indicate phylogenetic status (B, **Bacteria**; A, **Archaea**). Representatives of either domain of prokaryotic cells are known in each category. Most eukaryotes are obligate aerobes, but facultative aerobes (for example, yeast) and obligate anaerobes (for example, certain protozoa and fungi) are known.
[b]Listed are typical habitats of the example organism; many others could be listed.

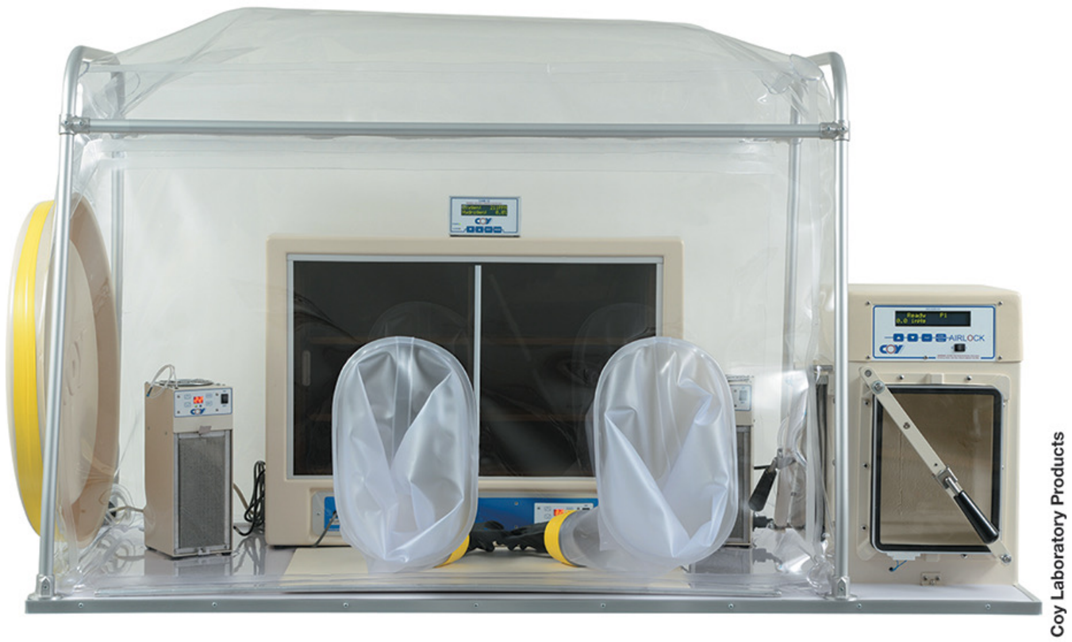
Culture Techniques for Aerobes and Anaerobes

- Aerobes need extensive aeration (**e.g.**, shaking, bubbling).

Culture Techniques for Aerobes and Anaerobes



- Anaerobes need oxygen excluded.
  - **reducing agents:** added to culture media to reduce oxygen to water
  - Flush or consume oxygen (**e.g.**, glove bag)



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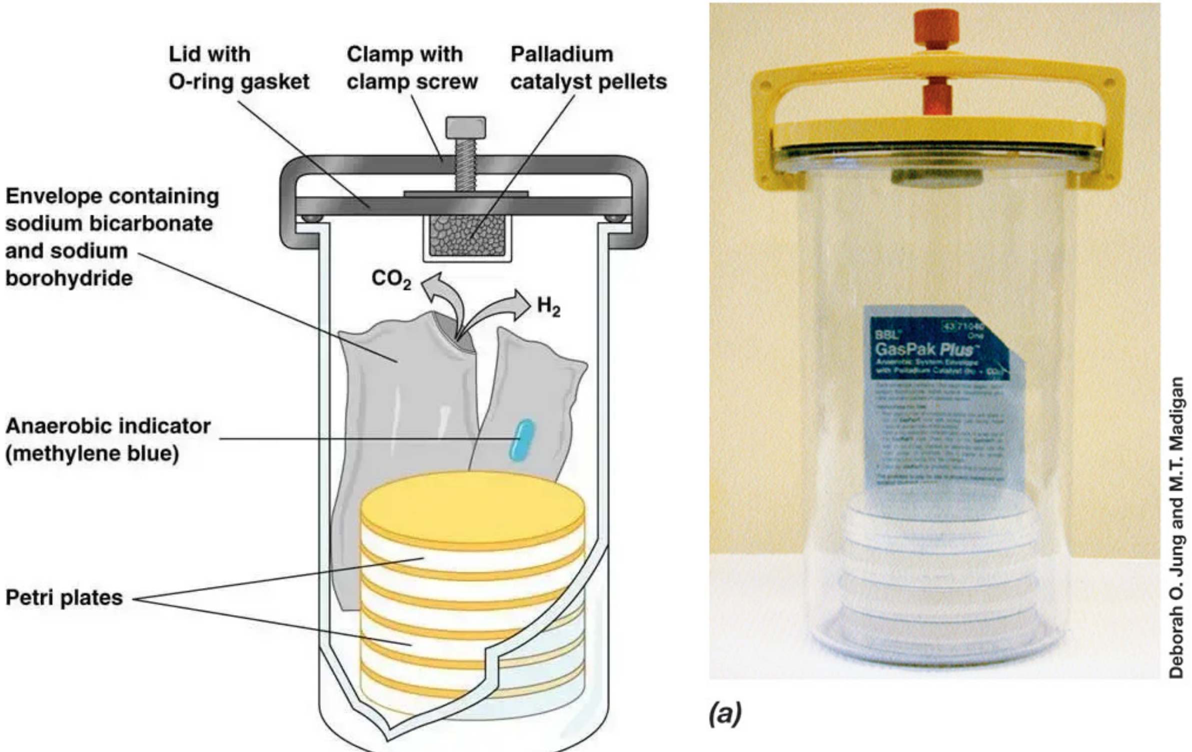
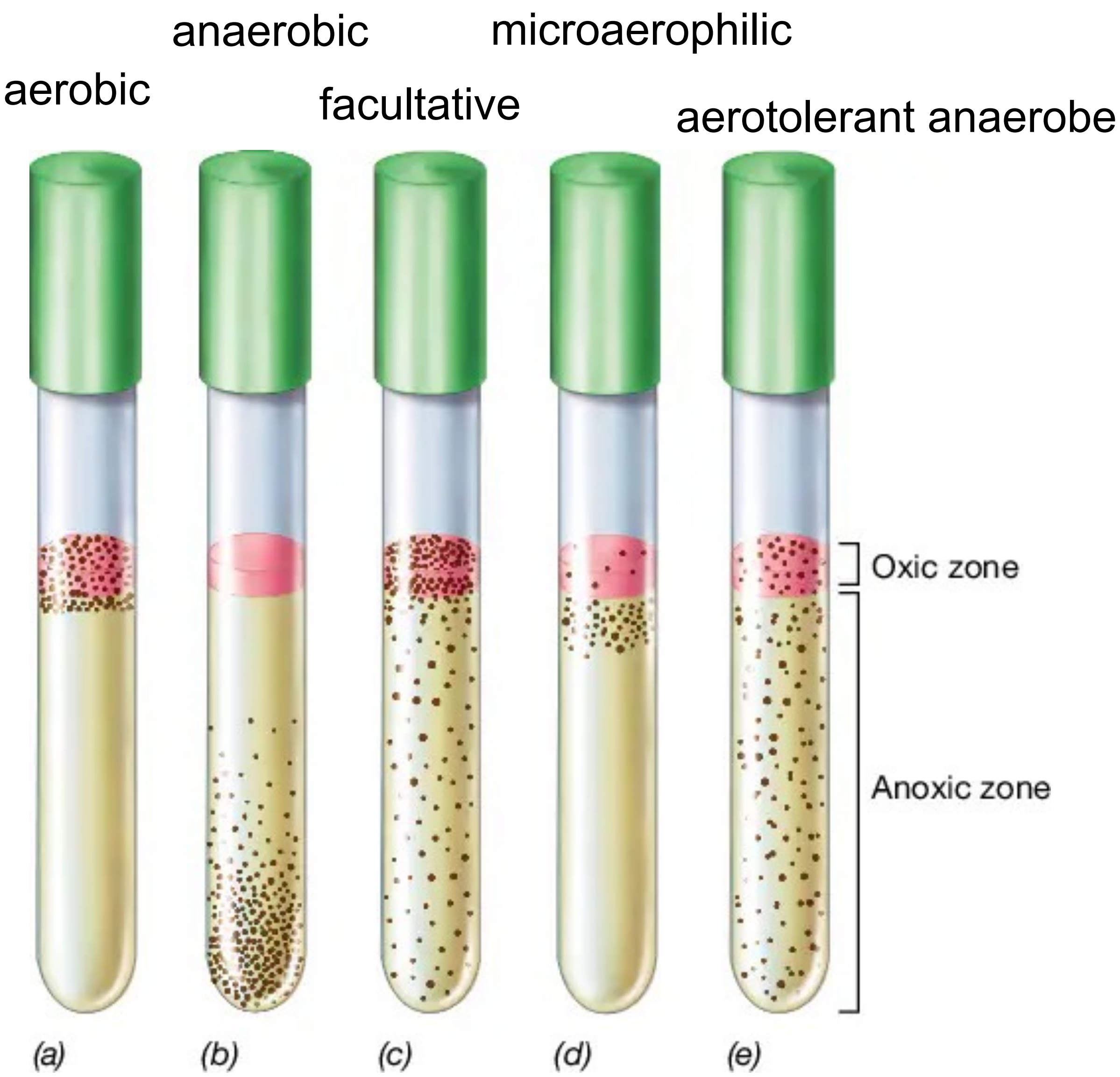


(a)

Culture Techniques for Aerobes and Anaerobes

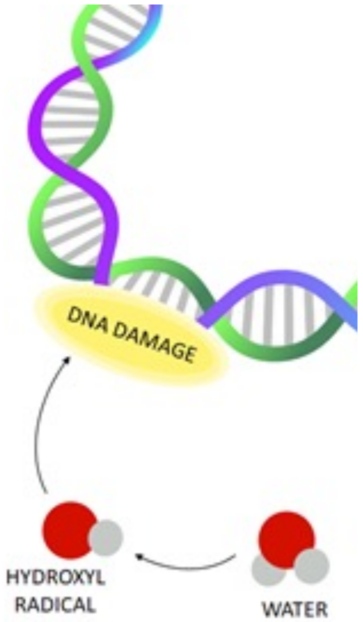


Why is oxygen toxic?

Why are anaerobic microorganisms inhibited in their growth or even killed by oxygen?

- Molecular oxygen (O_2) is not toxic.
- Exposure to oxygen yields toxic byproducts
  - **superoxide anion** (O_2^-)
  - **hydrogen peroxide** (H_2O_2)
  - **hydroxyl radical** ($OH\cdot$)

by-products of the reduction of O2 to H2O in respiration & electron carriers found in virtually all cells also can catalyse these reactions



Four-electron reduction of O_2 to H_2O by stepwise addition of electrons

Reactants	**Products**	
$O_2 + e^- \rightarrow$	O_2^-	(superoxide)
$O_2^- + e^- + 2\,H^+ \rightarrow$	H_2O_2	(hydrogen peroxide)
$H_2O_2 + e^- + H^+ \rightarrow$	H_2O + $OH\bullet$	(hydroxyl radical)
$OH\bullet + e^- + H^+ \rightarrow$	H_2O	(water)

Outcome:

$O_2 + 4\,e^- + 4\,H^+ \rightarrow 2\,H_2O$

Superoxide Dismutase and Other Enzymes That Destroy Toxic Oxygen

Keeping toxic oxygen molecules under control

Universal across life in O_2 environments

Superoxide Dismutase and Other Enzymes That Destroy Toxic Oxygen

Enzymes destroy O_2^- and H_2O_2

- Catalase and peroxidase convert H_2O_2 to O_2 and H_2O
- Superoxide dismutase converts 2 O_2^- to H_2O_2 and O_2
- **Superoxide reductase** in some strict anaerobes converts O_2^- to H_2O_2 without producing O_2

$$H_2O_2 + H_2O_2 \rightarrow 2\,H_2O + O_2$$

(*a*) **Catalase**

$$H_2O_2 + NADH + H^+ \rightarrow 2\,H_2O + NAD^+$$

(*b*) **Peroxidase**

$$O_2^- + O_2^- + 2\,H^+ \rightarrow H_2O_2 + O_2$$

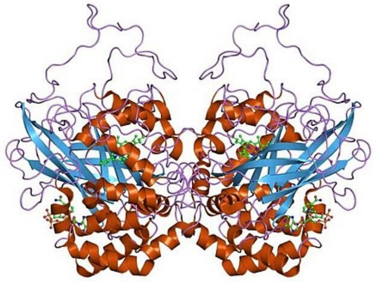
(*c*) **Superoxide dismutase**

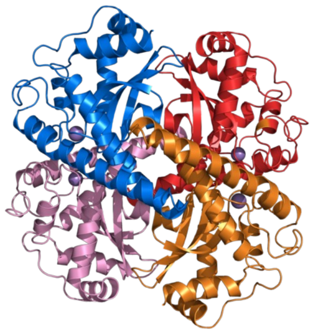
$$4\,O_2^- + 4\,H^+ \rightarrow 2\,H_2O + 3\,O_2$$

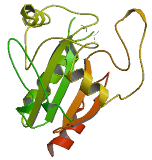
(*d*) **Superoxide dismutase/catalase in combination**

$$O_2^- + 2\,H^+ + \text{rubredoxin}_{reduced} \rightarrow H_2O_2 + \text{rubredoxin}_{oxidized}$$

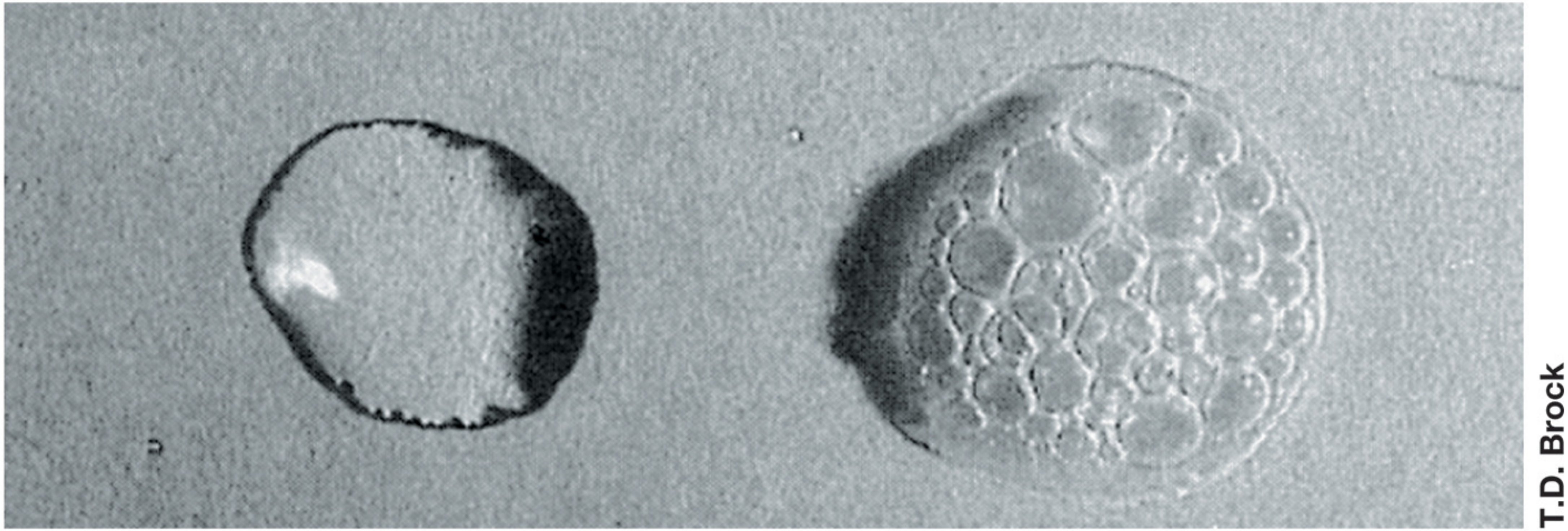
(*e*) **Superoxide reductase**







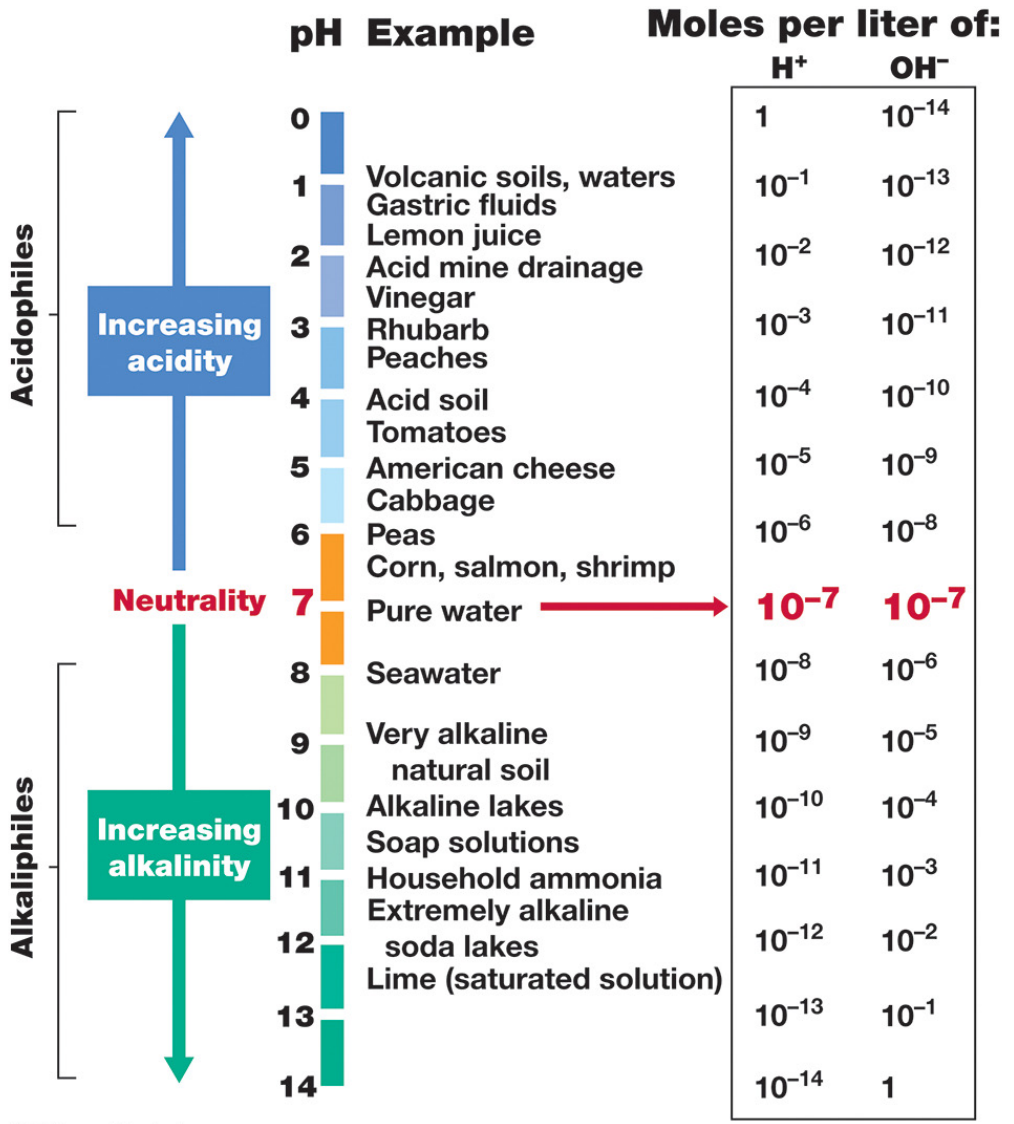
Presence of Catalase Test



$H_2O_2 + H_2O_2 \rightarrow 2\ H_2O + O_2$

Effects of pH on Microbial Growth

- pH expresses acidity or alkalinity of a solution.
- pH 7 = neutral
- **acidic** pH < 7, **alkaline** pH > 7
- Each microbe has a pH range ~2–3 pH units within which growth is possible.
- each organism shows a well-defined pH optimum
- Most natural environments are pH 3–9



Relationships of Microorganisms to pH

Physiological class (optima range)	Approximate pH optimum for growth	Example organism[a]
Neutrophile (pH > 5.5 and < 8)	7	**Escherichia coli**
Acidophile (pH < 5.5)	5	**Rhodopila globiformis**
	3	**Acidithiobacillus ferrooxidans**
	1	**Picrophilus oshimae**
Alkaliphile (pH ≥ 8)	8	**Chloroflexus aurantiacus**
	9	**Bacillus firmus**
	10	**Natronobacterium gregoryi**

[a]**Picrophilus** and **Natronobacterium** are **Archaea**; all others are **Bacteria**.

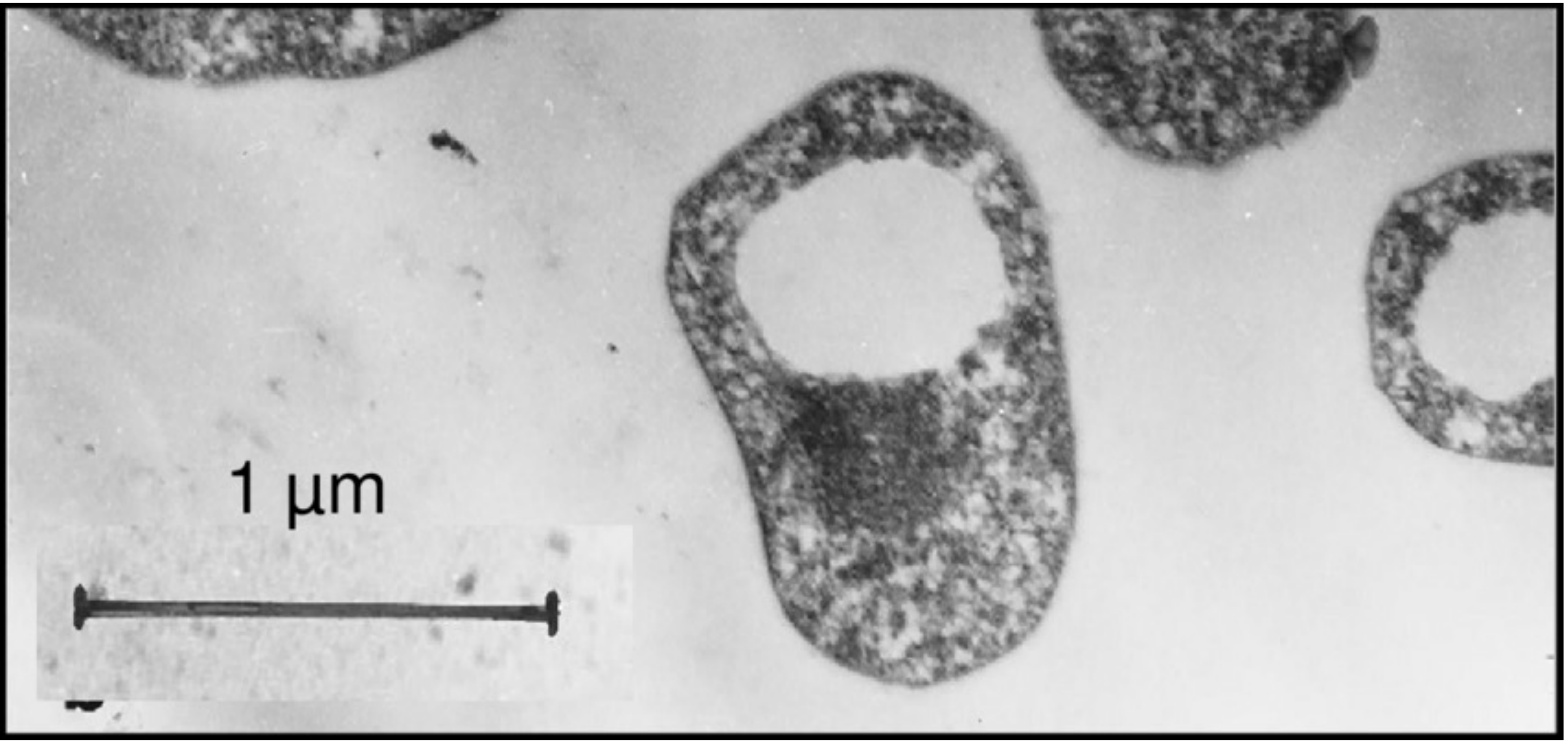
Although some microorganisms can live at very low or very high pH, the cell's internal pH remains near neutrality

Acidophiles

- Grow best at low pH (< 5.5)
- Different classes growing best at moderately acidic or very low pH
  - Many fungi and bacteria grow best at pH 5
  - Restricted number grow best below pH 3
  - Few grow best below pH 2
  - pH optima below 1 are extremely rare
  - Most acidophiles cannot grow at pH 7 and many cannot grow at pH values more than two units above their optimum

Acidophiles

- Governed by stability of cytoplasmic membrane
- At neutral pH, membranes of strong acidophiles lyse; protons required for stability



Picrophilus grows optimally at pH 0.7 and 60°C

Isolated from dry, extremely acidic soil (pH < 0.5) that was heated by solfataric gases to about 55C

- One of the smallest genomes
- High coding density in the genome
- Unknown DNA repair mechanism
- Large number of transport proteins: transmembrane proton gradient for transport

permanent huge ΔpH exists across the membrane of acidophiles

Alkaliphiles

- High pH optima for growth, pH ≥ 8
  - sometimes as high as pH 10
- Found in highly alkaline habitats: soda lakes and high-carbonate soils
- Used commercially (**e.g.**, secreted proteases and lipases that are added to laundry detergents to remove proteins and fats)
- Some have sodium (Na^+) motive force rather than proton motive force.

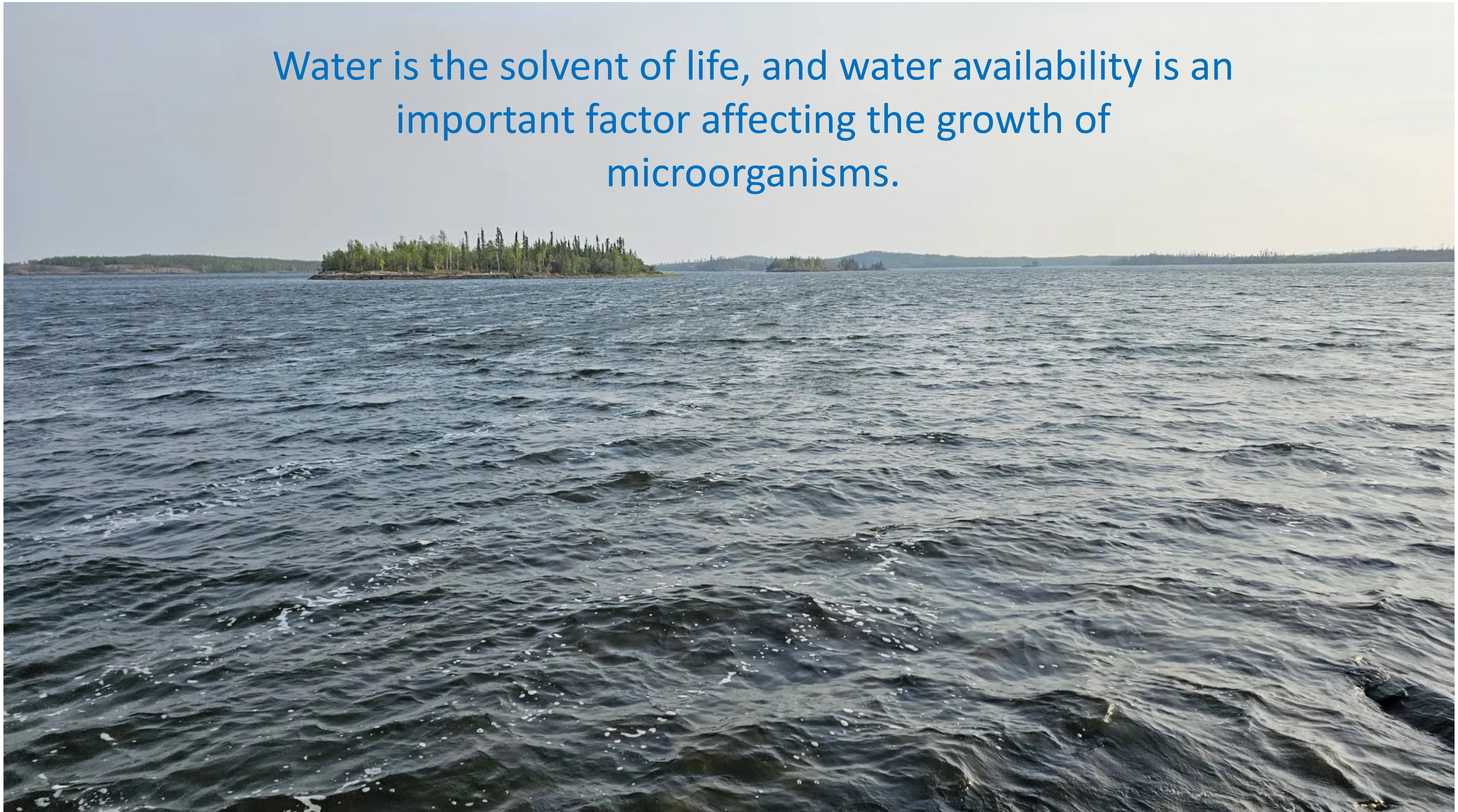
Bacillus firmus unusually broad range for growth, from pH 7.5 to 11

Natronobacterium gregoryi 20% and optimum pH of 10

Acidophiles & Alkaliphiles

- Cytoplasmic pH and Buffers
  - Optimal pH refers to extracellular only
  - The **intracellular** pH must stay relatively close to neutral (pH 5–9), consistent with macromolecule stability
    - Extreme acidophiles and alkaliphiles maintain cytoplasmic pH near neutrality.
  - Microbial culture media typically contain **buffers** to maintain constant pH.

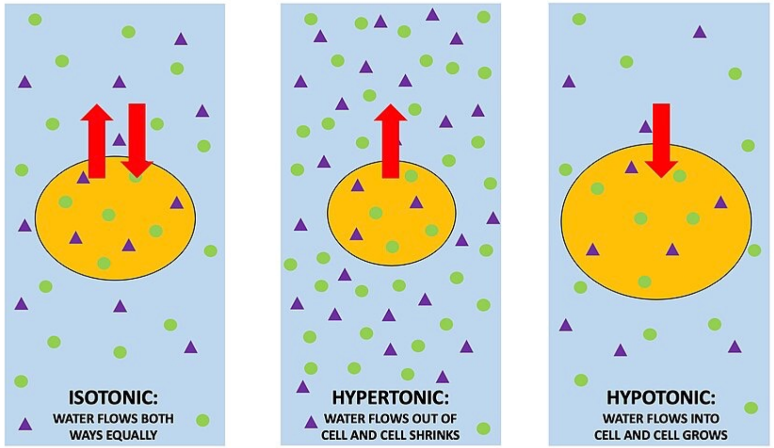
Osmolarity and Microbial Growth

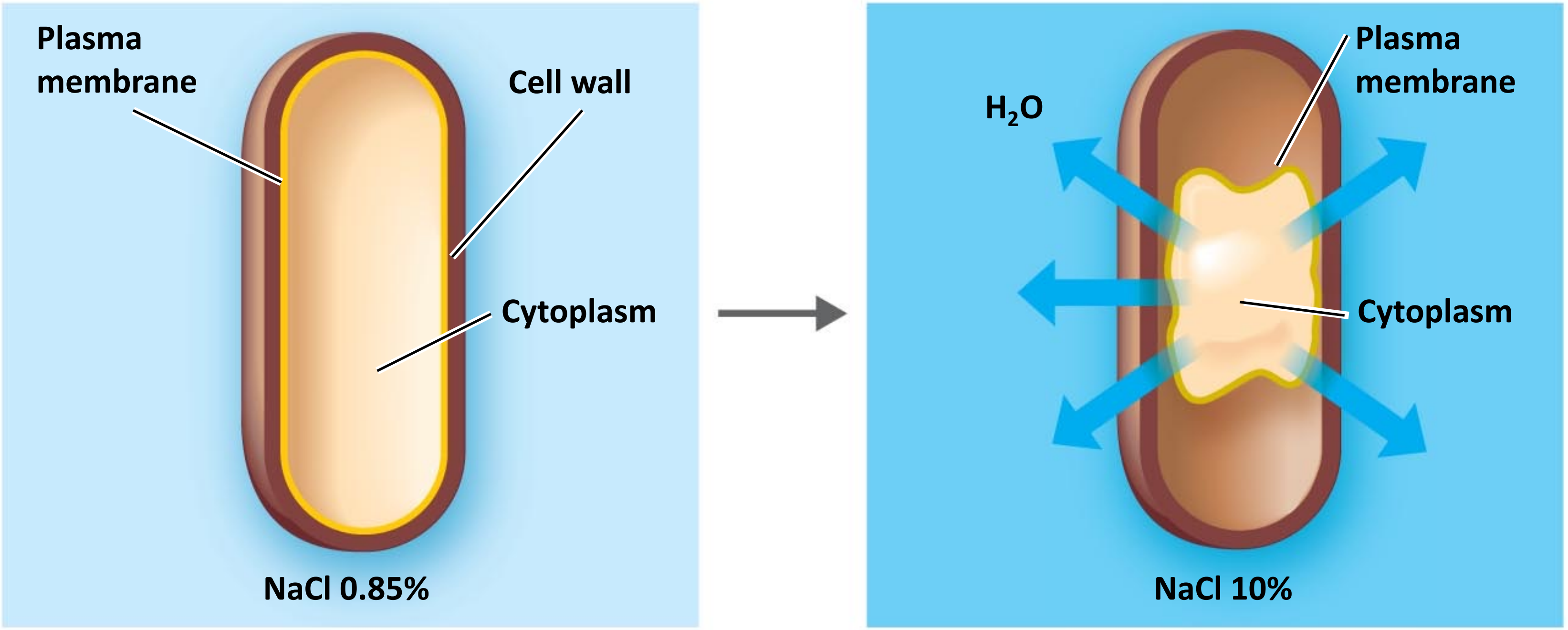


Osmolarity and Microbial Growth

Water is the solvent of life, and water availability is an important factor affecting the growth of microorganisms.

- Water availability depends on:
  - environmental moisture/dryness
  - concentration of solutes – bind water
- Water availability: Water activity (a_w)
  - varies from zero (no free water) to one (pure water)
- **Osmosis:** Water diffuses from high to low concentrations.
  - Positive vs. negative water balance
  - Cell cytoplasm typically have higher solute concentration than environment





(a) Cell in isotonic solution. Under these conditions, the solute concentration in the cell is equivalent to a solute concentration of 0.85% sodium chloride (NaCl).

(b) Plasmolyzed cell in hypertonic solution. If the concentration of solutes such as NaCl is higher in the surrounding medium than in the cell (the environment is hypertonic), water tends to leave the cell. Growth of the cell is inhibited.

Water Activity of Substances

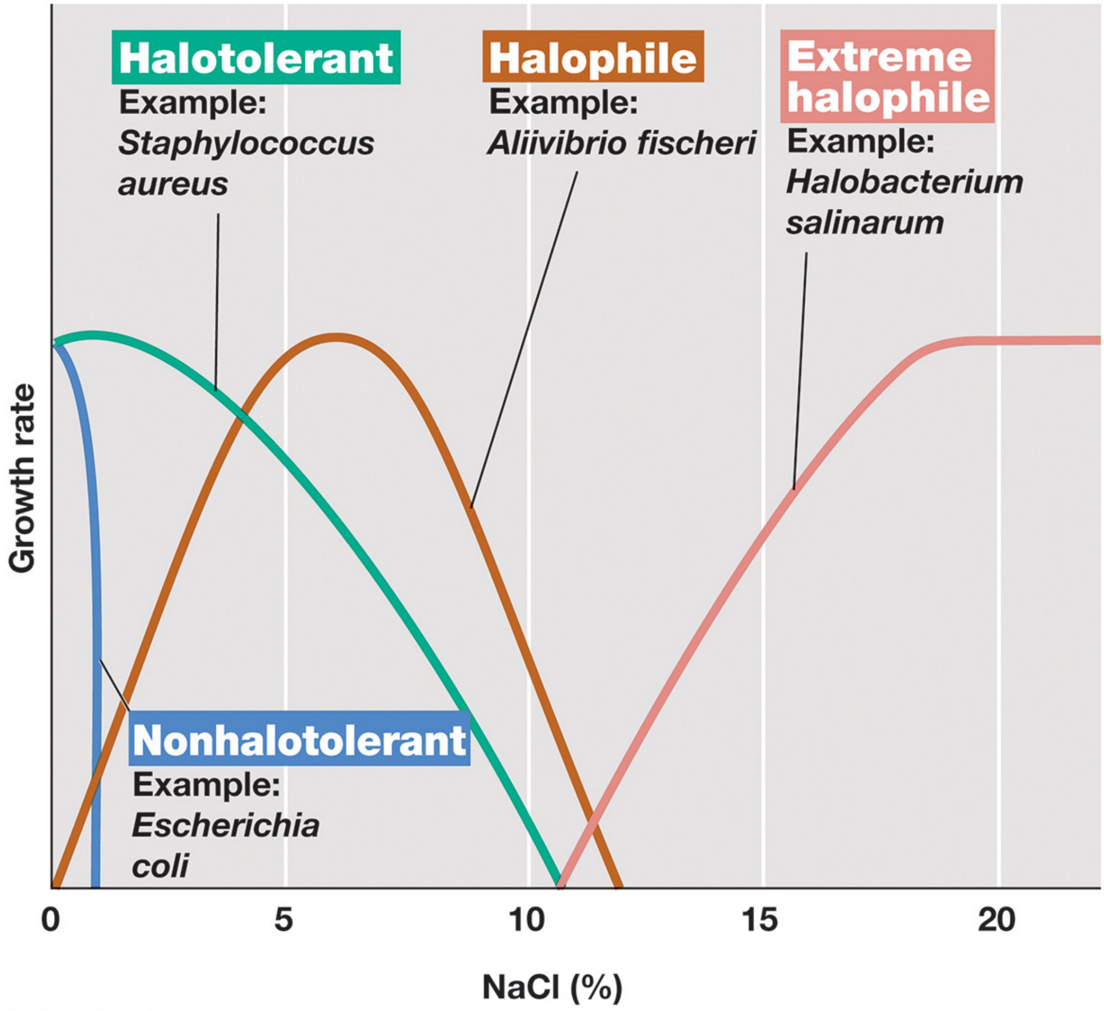
Water activity (a_w)	Material	Example organisms[a]
1.000	Pure water	**Caulobacter, Spirillum**
0.995	Human blood	**Streptococcus, Escherichia**
0.980	Seawater	**Pseudomonas, Vibrio**
0.950	Bread	Most gram-positive rods
0.900	Maple syrup, ham	Gram-positive cocci such as **Staphylococcus**
0.850	Salami	**Saccharomyces rouxii** (yeast)
0.800	Fruit cake, jams	**Zygosaccharomyces bailii** (yeast), **Penicillium** (fungus)
0.750	Salt lakes, salted fish	**Halobacterium, Halococcus**
0.700	Cereals, candy, dried fruit	**Xeromyces bisporus** and other xerophilic fungi

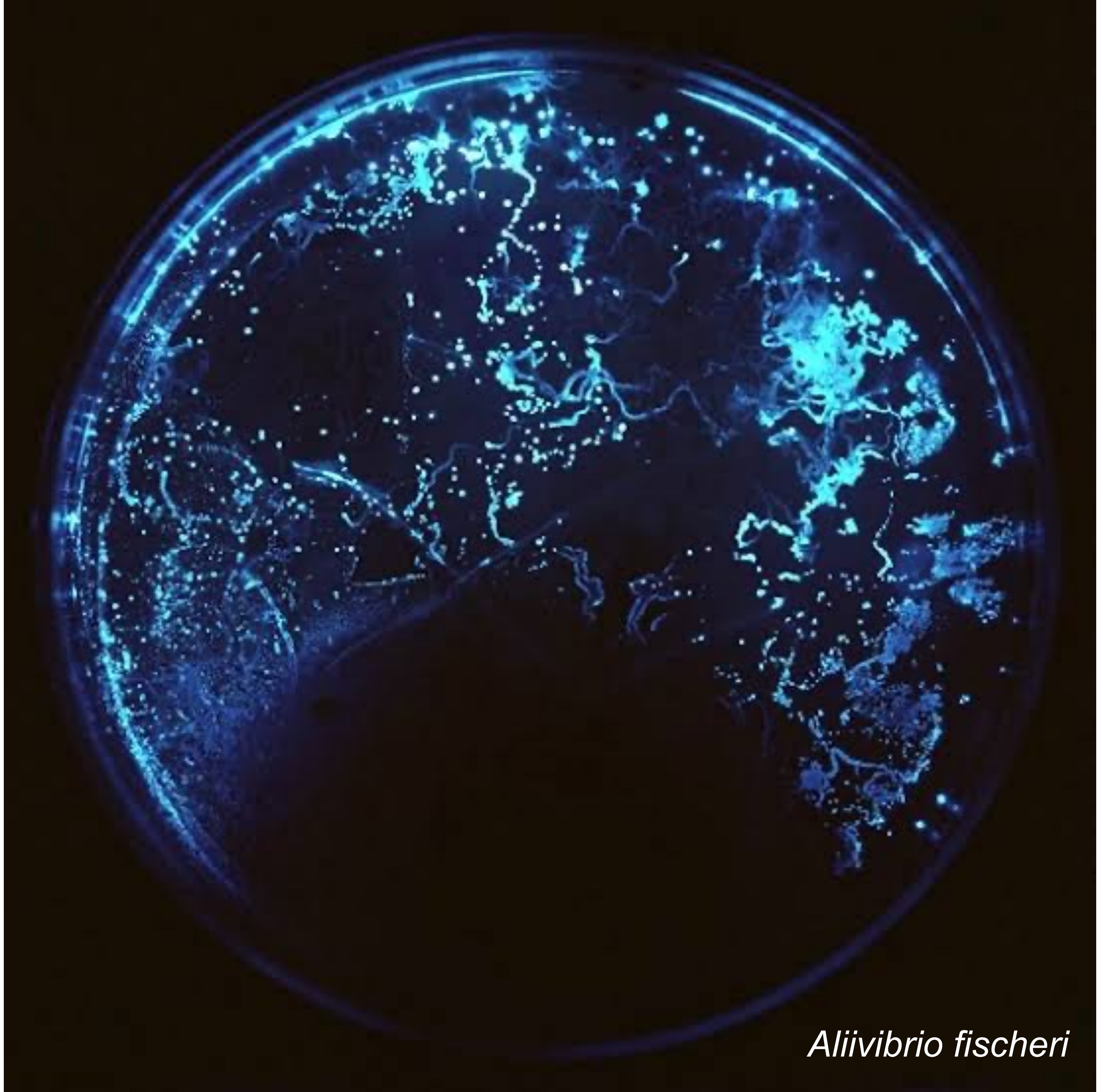
[a]Selected examples of **Bacteria** and **Archaea** or fungi capable of growth in culture media adjusted to the stated water activity.

Halophiles

- Seawater contains ~3% NaCl
  - Freshwater is <1%

- **Halophiles:** grow best at $a_w = 0.98$ (seawater); have a specific requirement for NaCl
- **Halotolerant:** tolerate some dissolved solutes but generally grow best in the absence of added solute
- **Extreme halophiles:** require very high levels (15-30%) of NaCl; often unable to grow at lower concentrations

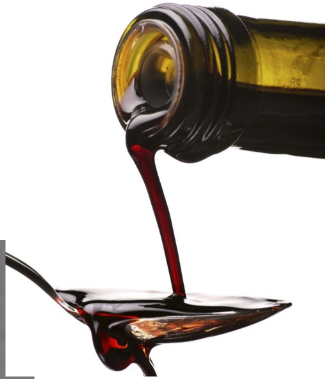



Aliivibrio fischeri

Related organisms

- Organisms able to live in environments high in sugar: Osmophiles

- Organisms able to live in very dry environments: Xerophiles



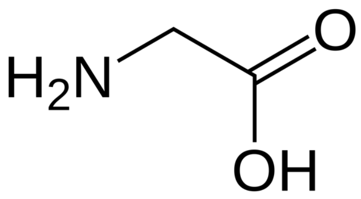
Wallemia sebi

Zygosaccharomyces rouxii

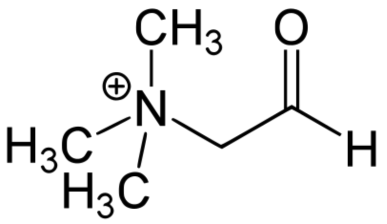
Compatible Solutes of Microorganisms

- Maintenance of positive water balance
- increase of internal solute concentration
- Pumping solutes into the cell from the environment
- Synthesizing a cytoplasmic solute
    - Must not inhibit biochemical pathways of the cell

Compatible solutes:
- highly water-soluble organic molecules
- sugars, alcohols, and amino acid derivatives

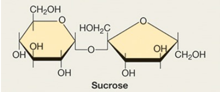
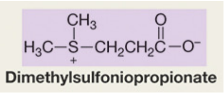
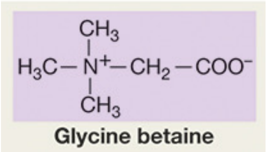
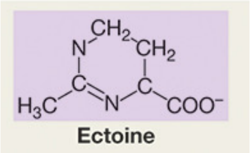


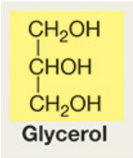
Glycine



Glycine betaine

Compatible Solutes of Microorganisms

Organism group and example	Major cytoplasmic compatible solute(s)	Minimum a_w for growth[c]
Most nonphototrophic **Bacteria** (**Escherichia**) and freshwater cyanobacteria (**Anabaena**)	Amino acids (mainly glutamate or proline[a])/sucrose, trehalose[b]	0.98 
Marine cyanobacteria (**Synechococcus**)	α-Glucosylglycerol[b]	0.92
Marine algae (**Phaeocystis**)	Mannitol,[b] various glycosides, dimethylsulfoniopropionate	0.92 
Halotolerant **Bacteria** (**Staphylococcus**)	Amino acids	0.90
Salt lake cyanobacteria (**Aphanothece**)	Glycine betaine	0.75 
Halophilic phototrophic purple **Bacteria** (**Halorhodospira**)	Glycine betaine, ectoine, trehalose[b]	0.75 

Organism group and example	Major cytoplasmic compatible solute(s)	Minimum a_w for growth[c]
Extremely halophilic **Archaea** (**Halobacterium**) and some **Bacteria** (**Salinibacter**)	KCl	0.75
Halophilic green algae (**Dunaliella**)	Glycerol	0.75 
Haloalkaliphilic **Archaea** (**Natrinema**)	KCl	0.68
Xerophilic and osmophilic yeasts (**Zygosaccharomyces**)	Glycerol	0.62
Xerophilic filamentous fungi (**Xeromyces**)	Glycerol	0.605

Why study extremophiles?