TEMPERATURE

READING: BOM-12

6.12 Effect of Temperature on Growth p. 157
6.13 Microbial Growth at Cold Temperatures p. 159
6.14 Microbial Growth at High Temperatures p. 162
9.11 Other Global Control Networks p. 240
27.1 Heat Sterilization p. 780
17.13 An Upper Temperature Limit for Microbial life p. 510
17.14 Adaptations to Life at High Temperature p. 511
6.12 Effect of Temperature on Growth
Cardinal Temperatures

The effect of temperature on growth rate (NOT viability) can be evaluated in terms of the Arrhenius equation for the rate of chemical reactions. The equation has been arranged to conform to the standard form of a straight line (y = mx+b).

The Arrhenius Plot displays the following "Cardinal Temperatures" and ranges:

- Minimum Temp.
- Maximum Temp.
- Optimum Temp.
- Total Growth Range
- Normal Growth Range

\[
\ln v = \left( -\frac{\Delta E^*}{R} \right) \left( \frac{1}{T} \right) + C
\]

\[
v = \text{rate} \\
\Delta E^* = \text{activation energy} \\
R = \text{gas constant} \\
T = \text{temperature in degrees Kelvin} \\
C = \text{constant}
\]

Here the curve responds to the effect of delta-T on delta-G according to the Gibbs free energy Eq.

The Normal Growth Range is the range within which the affect of temperature on growth rate mimics the effect of temperature on the rate of a pure chemical reaction. A temperature shift within this range elicits instantaneous alteration of rate. Shifts outside the Normal Range lead to new growth rate only after a lag. This implies that shifts within the Normal Range alter only the rate of chemical reactions by altering thermal energy of
reactants, and that cell composition is not altered. Shifts outside the Normal Range induce changes in gene expression and cell composition.

The characteristic form of the Arrhenius Plot is similar in virtually all Bacteria, though the values of the Cardinal Temperatures may vary considerably. For most bacteria, the Normal Range spans 30-40 C. Also, the Optimum Temp is NOT mid-way between Max.Temp. and Min. Temp. It is nearer to Max. than Min. Note that Fig. 6.16 and 6.17 are NOT Arrhenius Plots.

Interestingly, when E. coli is grown in laboratory culture, it is customarily grown at 37° C, which is the upper limit of the Normal Range, not the Optimum Temp. (39 °C).

Changes in medium composition often have small effects on the values of Cardinal temps. Frequently this includes a lowering of the Max. Temp. in minimal vs rich medium, implying thermolability of a biosynthetic process. In E. coli, for example, the Max. Temp. decreases 3 C (to 45 C) when it is grown in a medium lacking methionine. This effect is known to be caused by the thermolability of the biosynthetic enzyme homoserine trans-succinylase.² ³

Temperature Classes of Organisms

Temperature Classes are variously and approximately defined by reference to Cardinal Temperatures:

- **Psychrophile**: Minimum Temp. < 0 C
- **Mesophile**: Opt. Temp. 30 –40 C
- **Thermophile**: Max Temp. > 60 C
- **Hyperthermophile**: Max. Temp. > 90 C

6.13 Microbial Growth at Cold Temperatures

Cold Environments
Psychrophilic Microorganisms
Psychrotolerant Microorganisms
Molecular Adaptations to Psychrophily
Homeoviscous Adaptation

Lipid bilayer membranes exhibit a temperature-dependant phase transition between "Thermotropic Gel" and "Liquid Crystal". According to the "Fluid Mosaic" model of membrane structure and function, a membrane in a living cell must remain in the Liquid Crystal (fluid) phase.

<table>
<thead>
<tr>
<th>COLDER</th>
<th>WARMER</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;Thermotropic Gel&quot; phase transition between &quot;Melting Temp.&quot; (Tm)</td>
<td>&quot;Melting Temp.&quot; (Tm)</td>
</tr>
<tr>
<td>Activity of membrane proteins is impaired by loss of lateral mobility and/or failure to properly insert in membrane.</td>
<td>Membrane protein function is normal.</td>
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Homeoviscous Adaptation involves adjustment of membrane fluidity by altering the relative proportions of saturated vs unsaturated fatty acids used to synthesize membrane lipids. The cis double bonds in unsaturated fatty acids makes them "kinky". This reduces the hydrophobic interactions of adjacent lipid molecules in a monolayer, preventing close packing and increasing membrane fluidity. (Some bacteria increase fluidity by increasing the relative proportion of branched to unbranched fatty acids.)

For example, in Clostridium, thermophilic species have higher levels of saturated fatty acids compared to the levels in mesophilic species.

E. coli alters fatty acid composition of membrane lipids in response to temperature.

Freezing

6.14 Microbial Growth at High Temperatures
Thermal Environments
Hyperthermophiles in Hot Springs
Thermophiles
Protein Stability at High Temperatures

Random substitutions in a protein are much more likely to reduce thermal stability than to eliminate activity altogether.

It is fairly straightforward to generate single-step mutations that decrease the thermal stability of a protein at one end OR the other (but not both) of its Normal Range. The occurrence of such mutations in essential genes narrows the growth range of the organism, this is the basis of temperature-sensitive mutants.

Although reports of single-step mutations that increase thermal tolerance of an enzyme are fairly common, evidence for single-step mutations that increase thermal tolerance of an organism are exceedingly rare and may represent special cases. Increasing the thermal
tolerance of an organism presumably would require multiple independent mutations to increase thermal tolerance of ALL essential gene products.

Membrane Stability at High Temperatures
Thermophily and Biotechnology

9.11 Other Global Control Networks
Heat Shock Proteins
Heat Shock Response

Within several minutes following a shift from 37°C to 42°C, one observes increased levels of several dozen specific proteins. This so-called "Heat Shock Response" helps the cell recover from temporary exposure to temperatures above the normal growth range and is a classic example of gene regulation by reprogramming transcription using alternate RNA Polymerase Sigma Factors.

Nearly all organisms studied have a heat shock response generally similar to that of *E. coli*. Moreover, several of the heat shock proteins of *E. coli* are highly conserved, and demonstrate sequence homology with heat shock proteins of higher eukaryotes.

\( \sigma^{32} \) REGULON:

The alternate Sigma factor \( \sigma^{32} \) is synthesized constitutively, but ordinarily has a very short half-life. Heat shock decreases the degradation of \( \sigma^{32} \) and re-directs transcription to a family of promoter sequences upstream from the heat shock proteins (Hsp's).

Major classes of Hsp's:

- Hsp70: DnaK extreme sequence conservation, homologous to animal HSP70. dnaK null mutants are inviable above 42°C. Prevents aggregation of newly synthesized and unfolded proteins. Degradres \( \sigma^{32} \) allowing recovery from heat shock response.
- Hsp60: GroEL (Comprises 15% of total cell protein at 46°C; molecular chaperone
- Hsp10: Gro ES molecular chaperone
- various proteases

27.1 Heat Sterilization
Measuring Heat Sterilization
Endospores and Heat Sterilization
The Autoclave
Pasteurization

17.13 An Upper Temperature Limit for Microbial Life
What's the Upper limit?

17.14 Adaptations to Life at High Temperature
Stability of Monomers
The hydrolysis of low molecular weight metabolites may contribute to determining overall thermal tolerance of an organism. How significant is it that the *in vitro* 1/2 life of NAD and ATP are less than 30 minutes?

**Protein Folding and Thermostability**

Thermotolerant proteins have highly hydrophobic cores and high density of ionic interactions on surface.

**Cahperonins: Assisting Proteins to Remain in their Native State**

The thermosome of *Pyrodictium* allows survival for 1 hr. in autoclave (121°C) even though max. temp. for growth is 110°C.

**DNA Stability at High Temperatures: Solutes and Reverse Gyrase**

K-cyclic 2,3-bisphosphoglycerate reduces depurination.

Reverse DNA gyrase introduces + supercoils that stabilize DNA helix.

**Organic Polyamines**

**DNA Stability: DNA-Binding Proteins**

*Sac7d* (*Sulfolobus*) binds non-specifically in minor groove to raise Tm by 40°C

Archaeal "histone analogs" stabilize helix

**Lipid Stability**

dibiphytanyl tetraether lipid monolayer (see notes for membrane lecture)

**SSU rRNA Stability**
Discussion Questions:

1. What is an Arrhenius Plot? Draw an example. Label the axes and indicate the essential growth parameters ("cardinal temperatures").

2. How is the "Normal Growth Range" defined and what is the presumed physiological significance of this parameter?

3. What is the evidence for the requirement for heat-shock proteins at elevated temperatures?

4. What type of fatty acids predominate in membranes at higher temperatures?

5. What problems do cells experience at low temperatures?

6. Why are thermophilic bacteria interesting for biotechnology?

7. Why are the major molecular adaptations to thermophily?

8. What is the significance of "alternate sigma factors" to temperature physiology of bacteria?

9. What is the heat shock response?

10. Why are spontaneous mutants with a decreased normal growth range (with respect to temperature) more common than mutants with an expanded normal growth range?
References

1 The Swedish physical chemist Svante Arrhenius proposed (circa 1927) that bacteria originate on the planet Venus (where it is hotter than on Earth) and are continually transported to Earth via the solar wind.

Growth Rate of Escherichia coli at Elevated Temperatures: Reversible Inhibition of Homoserine Trans-Succinykinase.

Growth Rate of Escherichia coli at Elevated Temperatures: Limitation by Methionine.

4 Kristjansson, J. K. (1991)
Thermophilic Bacteria
CRC Press, Inc., Boca Raton, Fla.

Life at High Temperatures.

The Value of Basic Research: Discovery of Thermus aquaticus and Other Extreme Thermophiles
Genetics, 146; 1207-1210