Nutrition and Growth of Bacteria (page 1)

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Nutritional Requirements of Cells

Every organism must find in its environment all of the substances required for energy generation and cellular biosynthesis. The chemicals and elements of this environment that are utilized for bacterial growth are referred to as **nutrients** or **nutritional requirements**. Many bacteria can be grown the laboratory in **culture media** which are designed to provide all the essential nutrients in solution for bacterial growth. Bacteria that are symbionts or obligate intracellular parasites of other cells, usually eucaryotic cells, are (not unexpectedly) difficult to grow outside of their natural host cells. Whether the microbe is a mutualist or parasite, the host cell must ultimately provide the nutritional requirements of its resident.

Many bacteria can be identified in the environment by inspection or using genetic techniques, but attempts to isolate and grow them in artificial culture has been unsuccessful. This, in part, is the basis of the estimate that we may know less than one percent of all procaryotes that exist.

The Major Elements

At an elementary level, the nutritional requirements of a bacterium such as *E. coli*are revealed by the cell's elemental composition, which consists of C, H, O, N, S. P, K, Mg, Fe, Ca, Mn, and traces of Zn, Co, Cu, and Mo. These elements are found in the form of water, inorganic ions, small molecules, and macromolecules which serve either a structural or functional role in the cells. The general physiological functions of the elements are outlined in Table 1.

Table 1. Major elements, their sources and functions in bacterial cells.

Element	% of dry weight	Source	Function
Carbon	50	organic compounds or CO_2	Main constituent of cellular material
Oxygen	20	H_2O, organic compounds, CO_2, and O_2	Constituent of cell material and cell water; O_2 is electron acceptor in aerobic respiration
Nitrogen	14	NH_3, NO_3, organic compounds, N_2	Constituent of amino acids, nucleic acids nucleotides, and coenzymes
Hydrogen	8	H_2O, organic compounds, H_2	Main constituent of organic compounds and cell water
Phosphorus	3	inorganic phosphates (PO_4)	Constituent of nucleic acids, nucleotides, phospholipids, LPS, teichoic acids
Sulfur	1	SO_4, H_2S, S^o, organic sulfur compounds	Constituent of cysteine, methionine, glutathione, several coenzymes
Potassium	1	Potassium salts	Main cellular inorganic cation and cofactor for certain enzymes
Magnesium	0.5	Magnesium salts	Inorganic cellular cation, cofactor for certain enzymatic reactions

Calcium	0.5	Calcium salts	Inorganic cellular cation, cofactor for certain enzymes and a component of endospores
Iron	0.2	Iron salts	Component of cytochromes and certain nonheme iron-proteins and a cofactor for some enzymatic reactions

Trace Elements

Table 1 ignores the occurrence of trace elements in bacterial nutrition. **Trace elements** are metal ions required by certain cells in such small amounts that it is difficult to detect (measure) them, and it is not necessary to add them to culture media as nutrients. Trace elements are required in such small amounts that they are present as "contaminants" of the water or other media components. As metal ions, the trace elements usually act as cofactors for essential enzymatic reactions in the cell. One organism's trace element may be another's required element and vice-versa, but the usual cations that qualify as trace elements in bacterial nutrition are Mn, Co, Zn, Cu, and Mo.

Carbon and Energy Sources for Bacterial Growth

In order to grow in nature or in the laboratory, a bacterium must have an energy source, a source of carbon and other required nutrients, and a permissive range of physical conditions such as O_2 concentration, temperature, and pH. Sometimes bacteria are referred to as individuals or groups based on their patterns of growth under various chemical (nutritional) or physical conditions. For example, phototrophs are organisms that use light as an energy source; anaerobes are organisms that grow without oxygen; thermophiles are organisms that grow at high temperatures.

All living organisms require a source of energy. Organisms that use radiant energy (light) are called **phototrophs**. Organisms that use (oxidize) an organic form of carbon are called **heterotrophs** or **(chemo)heterotrophs**. Organisms that oxidize inorganic compounds are called **lithotrophs**.

The carbon requirements of organisms must be met by organic carbon (a chemical compound with a carbon-hydrogen bond) or by CO_2. Organisms that use organic carbon are **heterotrophs** and organisms that use CO_2 as a sole source of carbon for growth are called **autotrophs**.

Thus, on the basis of carbon and energy sources for growth four major nutritional types of procaryotes may be defined (Table 2).

Table 2. Major nutritional types of procaryotes

Nutritional Type	Energy Source	Carbon Source	Examples
Photoautotrophs	Light	CO_2	Cyanobacteria, some Purple and Green Bacteria
Photoheterotrophs	Light	Organic compounds	Some Purple and Green Bacteria
Chemoautotrophs or Lithotrophs (Lithoautotrophs)	Inorganic compounds, e.g. H_2, NH_3, NO_2, H_2S	CO_2	A few Bacteria and many Archaea

Chemoheterotrophs or Heterotrophs	Organic compounds	Organic compounds	Most Bacteria, some Archaea

Almost all eucaryotes are either photoautotrophic (e.g. plants and algae) or heterotrophic (e.g. animals, protozoa, fungi). Lithotrophy is unique to procaryotes and photoheterotrophy, common in the Purple and Green Bacteria, occurs only in a very few eucaryotic algae. Phototrophy has not been found in the Archaea, except for nonphotosynthetic light-driven ATP synthesis in the extreme halophiles.

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Growth Factors

This simplified scheme for use of carbon, either organic carbon or CO_2, ignores the possibility that an organism, whether it is an autotroph or a heterotroph, may require small amounts of certain organic compounds for growth because they are essential substances that the organism is unable to synthesize from available nutrients. Such compounds are called **growth factors**.

Growth factors are required in small amounts by cells because they fulfill specific roles in biosynthesis. The need for a growth factor results from either a blocked or missing metabolic pathway in the cells. Growth factors are organized into three categories.

1. **purines and pyrimidines**: required for synthesis of nucleic acids (DNA and RNA)

2. **amino acids**: required for the synthesis of proteins

3. **vitamins**: needed as coenzymes and functional groups of certain enzymes

Some bacteria (e.g. *E. coli*) do not require any growth factors: they can synthesize all essential purines, pyrimidines, amino acids and vitamins, starting with their carbon source, as part of their own intermediary metabolism. Certain other bacteria (e.g. *Lactobacillus*) require purines, pyrimidines, vitamins and several amino acids in order to grow. These compounds must be added in advance to culture media that are used to grow these bacteria. The growth factors are not metabolized directly as sources of carbon or energy, rather they are assimilated by cells to fulfill their specific role in metabolism. Mutant strains of bacteria that require some growth factor not needed by the wild type (parent) strain are referred to as **auxotrophs**. Thus, a strain of *E. coli* that requires the amino acid tryptophan in order to grow would be called a tryptophan auxotroph and would be designated *E. coli* ***trp-***

Figure 1. Cross-feeding between *Staphylococcus aureus* and *Haemophilus influenzae* growing on blood agar. © Gloria J. Delisle and Lewis Tomalty, Queens University, Kingston, Ontario, Canada. Licensed for use by ASM Microbe Library http://www.microbelibrary.org. *Haemophilus influenzae* was first streaked on to the blood agar plate followed by a cross streak with *Staphylococcus aureus*. *H. influenzae* is a fastidious bacterium which requires both hemin and NAD for growth. There is sufficient hemin in blood for growth of *Haemophilus,* but the medium is insufficient in NAD. *S. aureus* produces NAD in excess of its own needs and secretes it into the medium, which supports the growth of *Haemophilus* as satellite colonies.

Some vitamins that are frequently required by certain bacteria as growth factors are listed in Table 3. The function(s) of these vitamins in essential enzymatic reactions gives a clue why, if the cell cannot make the vitamin, it must be provided exogenously in order for growth to occur.

Table 3. Common vitamins required in the nutrition of certain bacteria.

Vitamin	Coenzyme form	Function
p-Aminobenzoic acid (PABA)	-	Precursor for the biosynthesis of folic acid
Folic acid	Tetrahydrofolate	Transfer of one-carbon units and required for synthesis of thymine, purine bases, serine, methionine and pantothenate
Biotin	Biotin	Biosynthetic reactions that require CO_2 fixation
Lipoic acid	Lipoamide	Transfer of acyl groups in oxidation of keto acids
Mercaptoethane-sulfonic acid	Coenzyme M	CH_4 production by methanogens

Nicotinic acid	NAD (nicotinamide adenine dinucleotide) and NADP	Electron carrier in dehydrogenation reactions
Pantothenic acid	Coenzyme A and the Acyl Carrier Protein (ACP)	Oxidation of keto acids and acyl group carriers in metabolism
Pyridoxine (B_6)	Pyridoxal phosphate	Transamination, deamination, decarboxylation and racemation of amino acids
Riboflavin (B_2)	FMN (flavin mononucleotide) and FAD (flavin adenine dinucleotide)	Oxidoreduction reactions
Thiamine (B_1)	Thiamine pyrophosphate (TPP)	Decarboxylation of keto acids and transaminase reactions
Vitamin B_{12}	Cobalamine coupled to adenine nucleoside	Transfer of methyl groups
Vitamin K	Quinones and napthoquinones	Electron transport processes

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Culture Media for the Growth of Bacteria

For any bacterium to be propagated for any purpose it is necessary to provide the appropriate biochemical and biophysical environment. The biochemical (nutritional) environment is made available as a **culture medium**, and depending upon the special needs of particular bacteria (as well as particular investigators) a large variety and types of culture media have been developed with different purposes and uses. Culture media are employed in the isolation and maintenance of pure cultures of bacteria and are also used for identification of bacteria according to their biochemical and physiological properties.

The manner in which bacteria are cultivated, and the purpose of culture media, varies widely. **Liquid media** are used for growth of pure batch cultures, while solidified media are used widely for the isolation of pure cultures, for estimating viable bacterial populations, and a variety of other purposes. The usual gelling agent for solid or **semisolid medium** is **agar**, a hydrocolloid derived from red algae. Agar is used because of its unique physical properties (it melts at 100°C and remains liquid until cooled to 40°C, the temperature at which it gels) and because it cannot be metabolized by most bacteria. Hence as a medium component it is relatively inert; it simply holds (gels) nutrients that are in aquaeous solution.

Types of Culture Media

Culture media may be classified into several categories depending on their composition or use.
A **chemically-defined (synthetic) medium** (Table 4a and 4b) is one in which the exact chemical composition is known. A **complex (undefined) medium** (Table 5a and 5b) is one in which the exact chemical constitution of the medium is not known. **Defined media** are usually composed of pure biochemicals off the shelf; complex media usually contain complex materials of biological origin such as

blood or milk or yeast extract or beef extract, the exact chemical composition of which is obviously undetermined. A defined medium is a **minimal medium** (Table 4a) if it provides only the exact nutrients (including any growth factors) needed by the organism for growth. The use of defined minimal media requires the investigator to know the exact nutritional requirements of the organisms in question. Chemically-defined media are of value in studying the minimal nutritional requirements of microorganisms, for enrichment cultures, and for a wide variety of physiological studies. Complex media usually provide the full range of growth factors that may be required by an organism so they may be more handily used to cultivate unknown bacteria or bacteria whose nutritional requirement are complex (i.e., organisms that require a lot of growth factors, known or unknown). Complex media are usually used for cultivation of bacterial pathogens and other fastidious bacteria.

Figure 2. *Legionella pneumophila.* Direct fluorescent antibody (DFA) stain of a patient respiratory tract specimen. © Gloria J. Delisle and Lewis Tomalty. Queens University, Kingston, Ontario, Canada. Licensed for use by ASM Microbe Library http://www.microbelibrary.org. In spite of its natural occurrence in water cooling towers and air conditioners, *Legionella* is a fastidious bacterium grown in the laboratory, which led to the long lag in identification of the first outbreak of Legionnaire's disease in Philadelphia in 1977. Had fluorescent antibody to the bacterium been available at that time, diagnosis could have been made as quickly as the time to prepare and view this slide.

Most pathogenic bacteria of animals, which have adapted themselves to growth in animal tissues, require complex media for their growth. Blood, serum and tissue extracts are frequently added to culture media for the cultivation of pathogens. Even so, for a few fastidious pathogens such as *Treponema pallidum*, the agent of syphilis, and *Mycobacterium leprae*, the cause of leprosy, artificial culture media and conditions have not been established. This fact thwarts the the ability to do basic research on these pathogens and the diseases that they cause.

Other concepts employed in the construction of culture media are the principles of selection and enrichment. A **selective medium** is one which has a component(s) added to it which will inhibit or prevent the growth of certain types or species of bacteria and/or promote the growth of desired species. One can also adjust the physical conditions of a culture medium, such as pH and temperature, to render it selective for organisms that are able to grow under these certain conditions.

A culture medium may also be a **differential medium** if allows the investigator to distinguish between different types of bacteria based on some observable trait in their pattern of growth on the medium. Thus a **selective, differential medium** for the isolation of *Staphylococcus aureus,* the most common bacterial pathogen of humans, contains a very high concentration of salt (which the staph will tolerate) that inhibits most other bacteria, mannitol as a source of fermentable sugar, and a pH indicator dye. From clinical specimens, only staph will grow. *S. aureus* is differentiated from *S. epidermidis* (a nonpathogenic component of the normal flora) on the basis of its ability to ferment mannitol. Mannitol-fermenting colonies *(S. aureus) produce* acid which reacts with the indicator dye forming a colored halo around the colonies; mannitol non-fermenters (*S. epidermidis*) use other non-fermentative substrates in the medium for growth and do not form a halo around their colonies.

An enrichment medium employs a slightly different twist. An **enrichment medium**(Table 5a and 5b) contains some component that permits the growth of specific types or species of bacteria, usually because they alone can utilize the component from their environment. However, an enrichment medium may have selective features. An enrichment medium for nonsymbiotic nitrogen-fixing bacteria omits a source of added nitrogen to the medium. The medium is inoculated with a potential source of these bacteria (e.g. a soil sample) and incubated in the atmosphere wherein the only source of nitrogen available is N_2. A selective enrichment medium (Table 5b) for growth of the extreme halophile (*Halococcus*) contains nearly 25 percent salt [NaCl], which is required by the extreme halophile and which inhibits the growth of all other procaryotes.

Table 4a. Minimal medium for the growth of *Bacillus megaterium.* An example of a chemically-defined medium for growth of a heterotrophic bacterium.

Component	Amount	Function of component
sucrose	10.0 g	C and energy source
K_2HPO_4	2.5 g	pH buffer; P and K source
KH_2PO_4	2.5 g	pH buffer; P and K source
$(NH_4)2HPO_4$	1.0 g	pH buffer; N and P source
$MgSO_4$ $7H_2O$	0.20 g	S and Mg^{++} source
$FeSO_4$ $7H_2O$	0.01 g	Fe^{++} source
$MnSO_4$ $7H_2O$	0.007 g	Mn^{++} Source
water	985 ml	
pH 7.0		

Table 4b. Defined medium (also an enrichment medium) for the growth of *Thiobacillus thiooxidans,* a lithoautotrophic bacterium.

Component	Amount	Function of component
NH_4Cl	0.52 g	N source
KH_2PO_4	0.28 g	P and K source
$MgSO_4$ $7H_2O$	0.25 g	S and Mg^{++} source
$CaCl_2$ $2H_2O$	0.07 g	Ca^{++} source
Elemental Sulfur	1.56 g	Energy source
CO_2	5%*	C source
water	1000 ml	
pH 3.0		

* Aerate medium intermittently with air containing 5% CO_2.

Table 5a. Complex medium for the growth of fastidious bacteria.

Component	Amount	Function of component
Beef extract	1.5 g	Source of vitamins and other growth factors
Yeast extract	3.0 g	Source of vitamins and other growth factors
Peptone	6.0 g	Source of amino acids, N, S, and P
Glucose	1.0 g	C and energy source
Agar	15.0 g	Inert solidifying agent
water	1000 ml	
pH 6.6		

Table 5b. Selective enrichment medium for growth of extreme halophiles.

Component	Amount	Function of component
Casamino acids	7.5 g	Source of amino acids, N, S and P
Yeast extract	10.0 g	Source of growth factors
Trisodium citrate	3.0 g	C and energy source
KCl	2.0 g	K^+ source
$MgSO_4$ 7 H_2O	20.0 g	S and Mg^{++} source
$FeCl_2$	0.023 g	Fe^{++} source
NaCl	250 g	Na^+ source for halophiles and inhibitory to nonhalophiles
water	1000 ml	
pH 7.4		

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Physical and Environmental Requirements for Microbial Growth

The procaryotes exist in nature under an enormous range of physical conditions such as O_2 concentration, Hydrogen ion concentration (pH) and temperature. The exclusion limits of life on the planet, with regard to environmental parameters, are always set by some microorganism, most often a procaryote, and frequently an Archaeon. Applied to all microorganisms is a vocabulary of terms used to describe their growth (ability to grow) within a range of physical conditions. A thermophile grows at high temperatures, an acidiphile grows at low pH, an osmophile grows at high solute concentration, and so on. This nomenclature will be employed in this section to describe the response of the procaryotes to a variety of physical conditions.

The Effect of Oxygen

Oxygen is a universal component of cells and is always provided in large amounts by H_2O. However, procaryotes display a wide range of responses to molecular oxygen O_2 (Table 6).

Obligate aerobes require O_2 for growth; they use O_2 as a final electron acceptor in aerobic respiration.

Obligate anaerobes (occasionally called **aerophobes**) do not need or use O_2 as a nutrient. In fact, O_2 is a toxic substance, which either kills or inhibits their growth. Obligate anaerobic procaryotes may live by fermentation, anaerobic respiration, bacterial photosynthesis, or the novel process of methanogenesis.

Facultative anaerobes (or **facultative aerobes**) are organisms that can switch between aerobic and anaerobic types of metabolism. Under anaerobic conditions (no O_2) they grow by fermentation or anaerobic respiration, but in the presence of O_2they switch to aerobic respiration.

Aerotolerant anaerobes are bacteria with an exclusively anaerobic (fermentative) type of metabolism but they are insensitive to the presence of O_2. They live by fermentation alone whether or not O_2 is present in

their environment.

Table 6. Terms used to describe O_2 Relations of Microorganisms.

		Environment	
Group	**Aerobic**	**Anaerobic**	**O_2 Effect**
Obligate Aerobe	Growth	No growth	Required (utilized for aerobic respiration)
Microaerophile	Growth if level not too high	No growth	Required but at levels below 0.2 atm
Obligate Anaerobe	No growth	Growth Toxic	
Facultative Anaerobe (Facultative Aerobe)	Growth	Growth	Not required for growth but utilized when available
Aerotolerant Anaerobe	Growth	Growth	Not required and not utilized

The response of an organism to O_2 in its environment depends upon the occurrence and distribution of various enzymes which react with O_2 and various oxygen radicals that are invariably generated by cells in the presence of O_2. All cells contain enzymes capable of reacting with O_2. For example, oxidations of flavoproteins by O_2invariably result in the formation of H_2O_2 (peroxide) as one major product and small quantities of an even more toxic free radical, superoxide or $O_2^{\cdot -}$. Also, chlorophyll and other pigments in cells can react with O_2 in the presence of light and generate singlet oxygen, another radical form of oxygen which is a potent oxidizing agent in biological systems.

In aerobes and aerotolerant anaerobes the potential for lethal accumulation of superoxide is prevented by the enzyme superoxide dismutase (Figure 1). All organisms which can live in the presence of O_2 (whether or not they utilize it in their metabolism) contain superoxide dismutase. Nearly all organisms contain the enzyme catalase, which decomposes H_2O_2. Even though certain aerotolerant bacteria such as the lactic acid bacteria lack catalase, they decompose H_2O_2 by means of peroxidase enzymes which derive electrons from $NADH_2$ to reduce peroxide to H_2O. Obligate anaerobes lack superoxide dismutase and catalase and/or peroxidase, and therefore undergo lethal oxidations by various oxygen radicals when they are exposed to O_2. See Figure 3 below.

All photosynthetic (and some nonphotosynthetic) organisms are protected from lethal oxidations of singlet oxygen by their possession of carotenoid pigments which physically react with the singlet oxygen radical and lower it to its nontoxic "ground" (triplet) state. Carotenoids are said to "quench" singlet oxygen radicals.

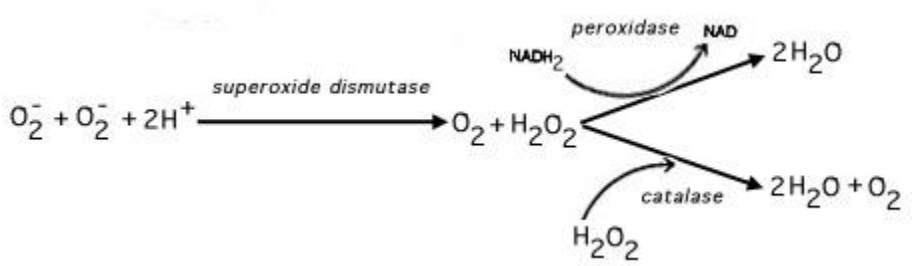

Figure 3. The action of superoxide dismutase, catalase and peroxidase. These enzymes detoxify oxygen

radicals that are inevitably generated by living systems in the presence of O_2. The distribution of these enzymes in cells determines their ability to exist in the presence of O_2

Table 7. Distribution of superoxide dismutase, catalase and peroxidase in procaryotes with different O_2 tolerances.

Group	Superoxide dismutase	Catalase	Peroxidase
Obligate aerobes and most facultative anaerobes (e.g. Enterics)	+	+	-
Most aerotolerant anaerobes (e.g. Streptococci)	+	-	+
Obligate anaerobes (e.g. Clostridia, Methanogens, Bacteroides)	-	-	-

The Effect of pH on Growth

The pH, or hydrogen ion concentration, $[H^+]$, of natural environments varies from about 0.5 in the most acidic soils to about 10.5 in the most alkaline lakes. Appreciating that pH is measured on a logarithmic scale, the $[H^+]$ of natural environments varies over a billion-fold and some microorganisms are living at the extremes, as well as every point between the extremes! Most free-living procaryotes can grow over a range of 3 pH units, about a thousand fold change in $[H^+]$. The range of pH over which an organism grows is defined by **three cardinal points**: the **minimum pH**, below which the organism cannot grow, the **maximum pH**, above which the organism cannot grow, and the **optimum pH**, at which the organism grows best. For most bacteria there is an orderly increase in growth rate between the minimum and the optimum and a corresponding orderly decrease in growth rate between the optimum and the maximum pH, reflecting the general effect of changing $[H^+]$ on the rates of enzymatic reaction (Figure 4).

Microorganisms which grow at an optimum pH well below neutrality (7.0) are called **acidophiles**. Those which grow best at neutral pH are called **neutrophiles** and those that grow best under alkaline conditions are called **alkaliphiles**. Obligate acidophiles, such as some *Thiobacillus* species, actually require a low pH for growth since their membranes dissolve and the cells lyse at neutrality. Several genera of Archaea, including *Sulfolobus* and *Thermoplasma*, are obligate acidophiles. Among eukaryotes, many fungi are acidophiles, but the champion of growth at low pH is the eucaryotic alga *Cyanidium* which can grow at a pH of 0.

In the construction and use of culture media, one must always consider the optimum pH for growth of a desired organism and incorporate **buffers** in order to maintain the pH of the medium in the changing milieu of bacterial waste products that accumulate during growth. Many pathogenic bacteria exhibit a relatively narrow range of pH over which they will grow. Most diagnostic media for the growth and identification of human pathogens have a pH near 7.

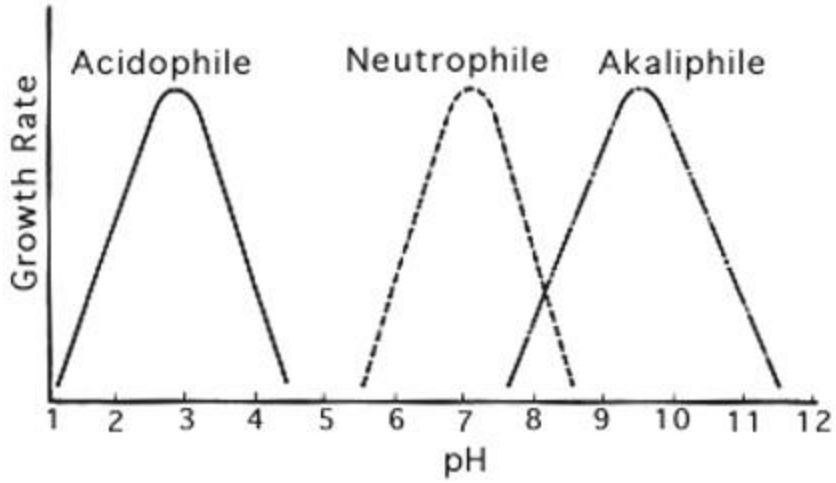

Figure 4. Growth rate vs pH for three environmental classes of procaryotes. Most free-living bacteria grow over a pH range of about three units. Note the symmetry of the curves below and above the optimum pH for growth.

Table 8. Minimum, maximum and optimum pH for growth of certain procaryotes.

Organism	Minimum pH	Optimum pH	Maximum pH
Thiobacillus thiooxidans	0.5	2.0-2.8	4.0-6.0
Sulfolobus acidocaldarius	1.0	2.0-3.0	5.0
Bacillus acidocaldarius	2.0	4.0	6.0
Zymomonas lindneri	3.5	5.5-6.0	7.5
Lactobacillus acidophilus	4.0-4.6	5.8-6.6	6.8
Staphylococcus aureus	4.2	7.0-7.5	9.3
Escherichia coli	4.4	6.0-7.0	9.0
Clostridium sporogenes	5.0-5.8	6.0-7.6	8.5-9.0
Erwinia caratovora	5.6	7.1	9.3
Pseudomonas aeruginosa	5.6	6.6-7.0	8.0
Thiobacillus novellus	5.7	7.0	9.0
Streptococcus pneumoniae	6.5	7.8	8.3
Nitrobacter sp	6.6	7.6-8.6	10.0

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The Effect of Temperature on Growth

Microorganisms have been found growing in virtually all environments where there is liquid water, regardless of its temperature. In 1966, Professor Thomas D. Brock, then at Indiana

University, made the amazing discovery in boiling hot springs of Yellowstone National Park that bacteria were not just surviving there, they were growing and flourishing. Brock's discovery of thermophilic bacteria, archaea and other "extremophiles" in Yellowstone is summarized for the general public in an article at this web site. See Life at High Temperatures.

Subsequently, procaryotes have been detected growing around black smokers and hydrothermal vents in the deep sea at temperatures at least as high as 120 degrees. Microorganisms have been found growing at very low temperatures as well. In supercooled solutions of H_2O as low as -20 degrees, certain organisms can extract water for growth, and many forms of life flourish in the icy waters of the Antarctic, as well as household refrigerators, near 0 degrees.

A particular microorganism will exhibit a range of temperature over which it can grow, defined by three cardinal points in the same manner as pH (Figure 6, cf. Figure 4). Considering the total span of temperature where liquid water exists, the procaryotes may be subdivided into several subclasses on the basis of one or another of their cardinal points for growth. For example, organisms with an optimum temperature near 37 degrees (the body temperature of warm-blooded animals) are called **mesophiles**. Organisms with an optimum T between about 45 degrees and 70 degrees are **thermophiles**. Some Archaea with an optimum T of 80 degrees or higher and a maximum T as high as 115 degrees, are now referred to as **extreme thermophiles** or **hyperthermophiles**. The cold-loving organisms are **psychrophiles** defined by their ability to grow at 0 degrees. A variant of a psychrophile (which usually has an optimum T of 10-15 degrees) is a **psychrotroph**, which grows at 0 degrees but displays an optimum T in the mesophile range, nearer room temperature. Psychrotrophs are the scourge of food storage in refrigerators since they are invariably brought in from their mesophilic habitats and continue to grow in the refrigerated environment where they spoil the food. Of course, they grow slower at 2 degrees than at 25 degrees. Think how fast milk spoils on the counter top versus in the refrigerator.

Psychrophilic bacteria are adapted to their cool environment by having largely unsaturated fatty acids in their plasma membranes. Some psychrophiles, particularly those from the Antarctic have been found to contain polyunsaturated fatty acids, which generally do not occur in procaryotes. The degree of unsaturation of a fatty acid correlates with its solidification T or thermal transition stage (i.e., the temperature at which the lipid melts or solidifies); unsaturated fatty acids remain liquid at low T but are also denatured at moderate T; saturated fatty acids, as in the membranes of thermophilic bacteria, are stable at high temperatures, but they also solidify at relatively high T. Thus, saturated fatty acids (like butter) are solid at room temperature while unsaturated fatty acids (like safflower oil) remain liquid in the refrigerator. Whether fatty acids in a membrane are in a liquid or a solid phase affects the fluidity of the membrane, which directly affects its ability to function. Psychrophiles also have enzymes that continue to function, albeit at a reduced rate, at temperatures at or near 0 degrees. Usually, psychrophile proteins and/or membranes, which adapt them to low temperatures, do not function at the body temperatures of warm-blooded animals (37 degrees) so that they are unable to grow at even moderate temperatures.

Thermophiles are adapted to temperatures above 60 degrees in a variety of ways. Often thermophiles have a high G + C content in their DNA such that the melting point of the DNA (the temperature at which the strands of the double helix separate) is at least as high as the

organism's maximum T for growth. But this is not always the case, and the correlation is far from perfect, so thermophile DNA must be stabilized in these cells by other means. The membrane fatty acids of thermophilic bacteria are highly saturated allowing their membranes to remain stable and functional at high temperatures. The membranes of hyperthermophiles, virtually all of which are Archaea, are not composed of fatty acids but of repeating subunits of the C5 compound, phytane, a branched, saturated, "isoprenoid" substance, which contributes heavily to the ability of these bacteria to live in superheated environments. The structural proteins (e.g. ribosomal proteins, transport proteins (permeases) and enzymes of thermophiles and hyperthermophiles are very heat stable compared with their mesophilic counterparts. The proteins are modified in a number of ways including dehydration and through slight changes in their primary structure, which accounts for their thermal stability.

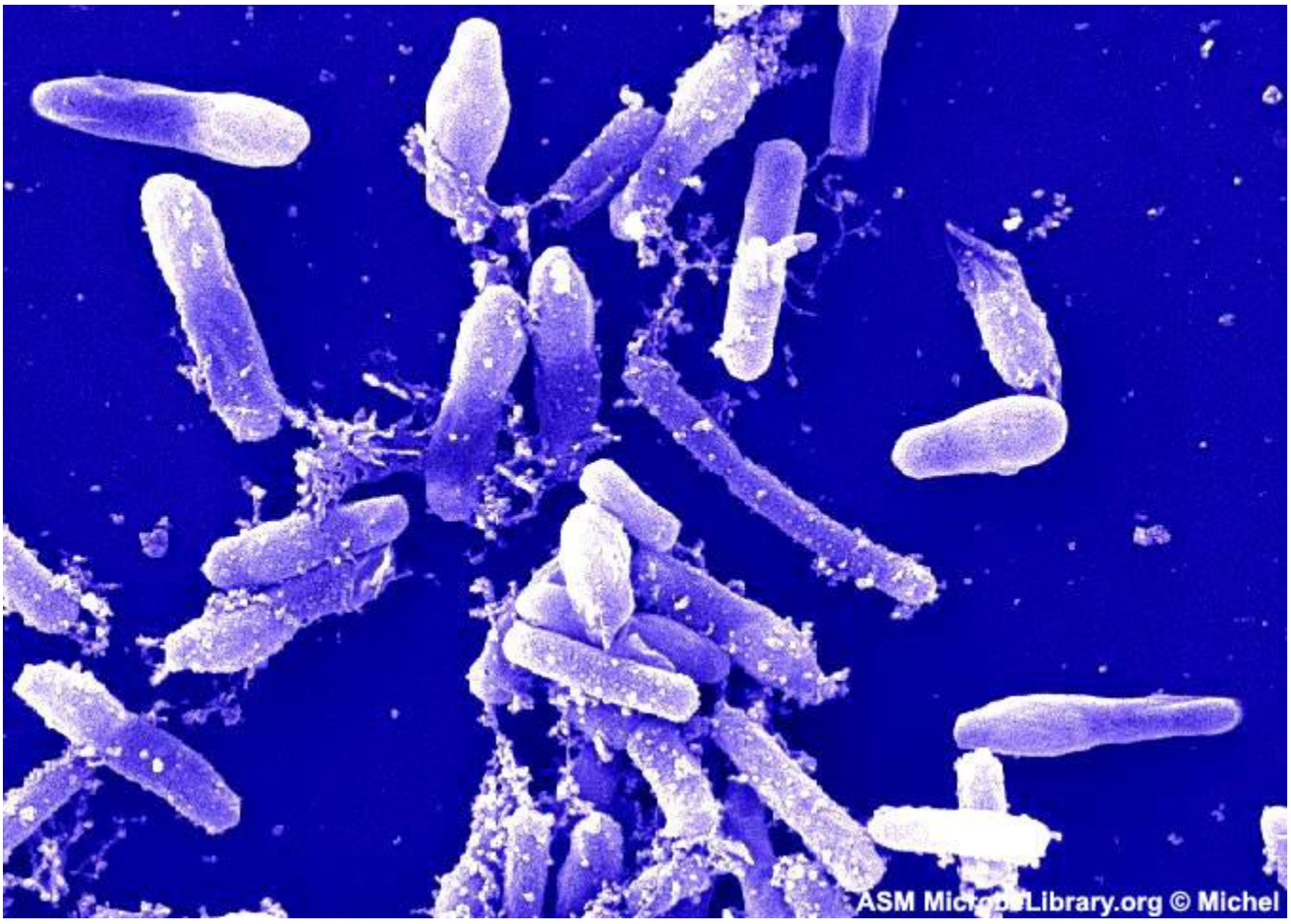

Figure 5. SEM of a thermophilic *Bacillus* species isolated from a compost pile at 55ºC. © Frederick C. Michel. The Ohio State University - OARDC, Wooster, Ohio. Licensed for use by ASM Microbe Library http://www.microbelibrary.org. The rods are 3-5 microns in length and 0.5 to 1 micron in width with terminal endospores in a slightly-swollen sporangium.

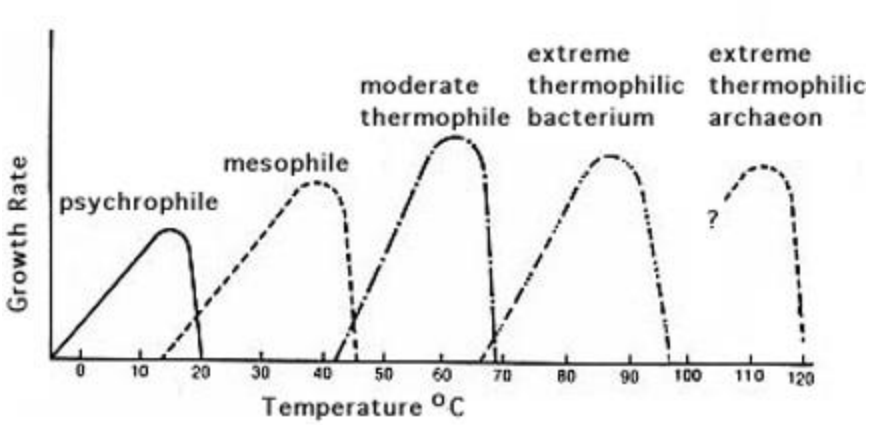

Figure 6 (below). Growth rate vs temperature for five environmental classes of procaryotes. Most procaryotes will grow over a temperature range of about 30 degrees. The curves exhibit three cardinal points: minimum, optimum and maximum temperatures for growth. There is a steady increase in growth rate between the minimum and optimum temperatures, but slightly past the optimum a critical thermolabile cellular event occurs, and the growth rates plunge rapidly as the maximum T is approached. As expected and as predicted by T.D. Brock, life on earth, with regard to temperature, exists wherever water remains in a liquid state. Thus, psychrophiles grow in solution wherever water is supercooled below 0 degrees; and extreme thermophilic archaea (hyperthermophiles) have been identified growing near deep-sea thermal vents at temperatures up to 120 degrees. Theoretically, the bar can be pushed to even higher temperatures.

Table 9. Terms used to describe microorganisms in relation to temperature requirements for growth.

Temperature for growth (degrees C)

Group	Minimum	Optimum	Maximum	Comments
Psychrophile	Below 0	10-15	Below 20	Grow best at relatively low T
Psychrotroph	0	15-30	Above 25	Able to grow at low T but prefer moderate T
Mesophile	10-15	30-40	Below 45	Most bacteria esp. those living in association with warm-blooded animals
Thermophile*	45	50-85	Above 100 (boiling)	Among all thermophiles is wide variation in optimum and maximum T

* For "degrees" of thermophily see text and graphs above

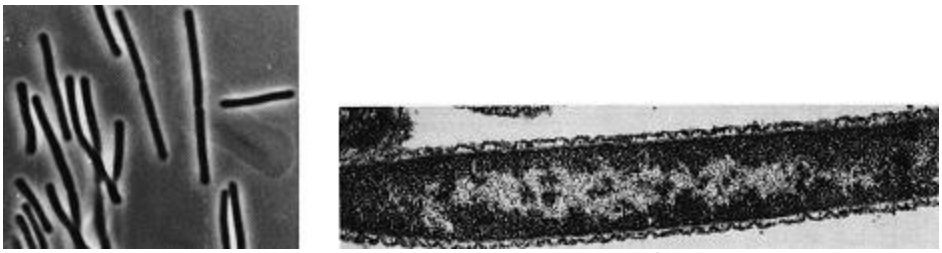

Figure 7. ***Thermus aquaticus, the*** **thermophilic bacterium that is the source of taq polymerase. L wet mount; R electron micrograph. T.D. Brock. Life at High Temperatures.**

Table 10a. Minimum, maximum and optimum temperature for growth of certain bacteria and archaea.

	Temperature for growth (degrees C)		
Bacterium	**Minimum**	**Optimum**	**Maximum**
Listeria monocytogenes	1	30-37	45
Vibrio marinus	4	15	30
Pseudomonas maltophilia	4	35	41
Thiobacillus novellus	5	25-30	42
Staphylococcus aureus	10	30-37	45
Escherichia coli	10	37	45
Clostridium kluyveri	19	35	37
Streptococcus pyogenes	20	37	40
Streptococcus pneumoniae	25	37	42
Bacillus flavothermus	30	60	72
Thermus aquaticus	40	70-72	79
Methanococcus jannaschii	60	85	90
Sulfolobus acidocaldarius	70	75-85	90
Pyrobacterium brockii	80	102-105	115

Table 10b. Optimum growth temperature of some procaryotes.

Genus and species	**Optimal growth temp (degrees C)**
Vibrio cholerae	18-37
Photobacterium phosphoreum	20
Rhizobium leguminosarum	20
Streptomyces griseus	25
Rhodobacter sphaeroides	25-30
Pseudomonas fluorescens	25-30
Erwinia amylovora	27-30
Staphylococcus aureus	30-37
Escherichia coli	37

Mycobacterium tuberculosis	37
Pseudomonas aeruginosa	37
Streptococcus pyogenes	37
Treponema pallidum	37
Thermoplasma acidophilum	59
Thermus aquaticus	70
Bacillus caldolyticus	72
Pyrococcus furiosus	100

Table 10c. Hyperthermophilic Archaea.

	Temperature for growth (degrees C)			
Genus	**Minimum**	**Optimum**	**Maximum**	**Optimum pH**
Sulfolobus	55	75-85	87	2-3
Desulfurococcus	60	85	93	6
Methanothermus	60	83	88	6-7
Pyrodictium	82	105	113	6
Methanopyrus	85	100	110	7

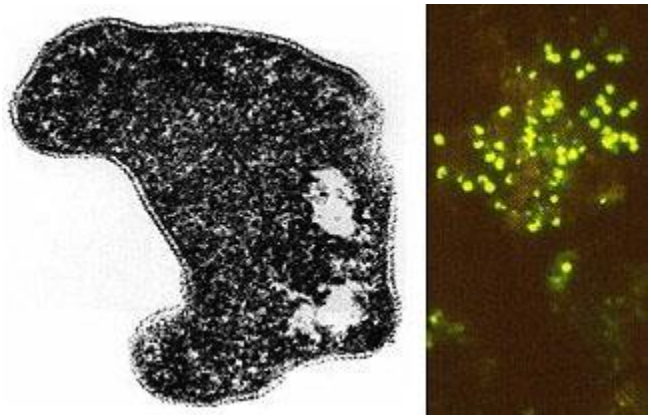

Figure 8. *Sulfolobus acidocaldarius* is an extreme thermophile and an acidophile found in geothermally-heated acid springs, mud pots and surface soils with temperatures from 60 to 95 degrees C, and a pH of 1 to 5. Left: Electron micrograph of a thin section (85,000X). Under the electron microscope the organism appears as irregular spheres which are often lobed. Right: Fluorescent photomicrograph of cells attached to a sulfur crystal. Fimbrial-like appendages have been observed on the cells attached to solid surfaces such as sulfur crystals. T.D. Brock. Life at High Temperatures.

Nutrition and Growth of Bacteria (page 6)

(This chapter has 6 pages)

Water Availability

Water is the solvent in which the molecules of life are dissolved, and the availability of water is therefore a critical factor that affects the growth of all cells. The availability of water for a cell depends upon its presence in the atmosphere (relative humidity) or its presence in solution or a substance (**water activity**). The water activity (A_w) of pure H_2O is 1.0 (100% water). Water activity is affected by the presence of solutes such as salts or sugars, that are dissolved in the water. The higher the solute concentration of a substance, the lower is the water activity and vice-versa. Microorganisms live over a range of A_w from 1.0 to 0.7. The A_w of human blood is 0.99; seawater = 0.98; maple syrup = 0.90; Great Salt Lake = 0.75. Water activities in agricultural soils range between 0.9 and 1.0.

The only common solute in nature that occurs over a wide concentration range is salt [NaCl], and some microorganisms are named based on their growth response to salt. Microorganisms that require some NaCl for growth are **halophiles**. **Mild halophiles** require 1-6% salt, **moderate halophiles** require 6-15% salt; **extreme halophiles** that require 15-30% NaCl for growth are found among the archaea. Bacteria that are able to grow at moderate salt concentrations, even though they grow best in the absence of NaCl, are called **halotolerant**. Although halophiles are "osmophiles" (and halotolerant organisms are "osmotolerant") the term **osmophiles**is usually reserved for organisms that are able to live in environments high in sugar. Organisms which live in dry environments (made dry by lack of water) are called **xerophiles**.

The concept of lowering water activity in order to prevent bacterial growth is the basis for preservation of foods by drying (in sunlight or by evaporation) or by addition of high concentrations of salt or sugar.

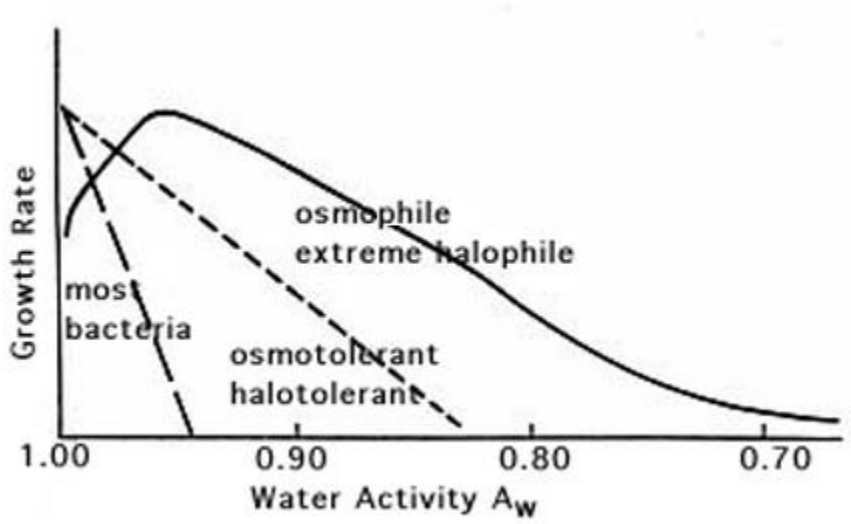

Figure 9. Growth rate vs osmolarity for different classes of procaryotes. Osmolarity is determined by solute concentration in the environment. Osmolarity is inversely related to water activity (A_w), which is more like a measure of the concentration of water (H_2O) in a solution. Increased solute concentration means increased osmolarity and decreased A_w. From left to right the graph shows the growth rate of a normal (nonhalophile) such as *E. coli* or *Pseudomonas,* the growth rate of a halotolerant bacterium such as *Staphylococcus aureus, and* the growth rate of an extreme halophile such as the archaean *Halococcus. Note* that a true halophile grows best at salt concentrations where most bacteria are inhibited.

Table 11. Limiting water activities (A_w) for growth of certain procaryotes.

Organism	**Minimum A_w for growth**
Caulobacter	1.00
Spirillum	1.00
Pseudomonas	.91
Salmonella/E. coli	.91
Lactobacillus	.90
Bacillus	.90
Staphylococcus	.85
Halococcus	.75

END OF CHAPTER