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Introduction to bacterial physiology and metabolism

The biosphere has been shaped both by physical events and by interactions with the organisms that occupy it. Among living organisms, prokaryotes are much more metabolically diverse than eukaryotes and can also thrive under a variety of extreme conditions where eukaryotes cannot. This is possible because of the wealth of genes, metabolic pathways and molecular processes that are unique to prokaryotic cells. For this reason, prokaryotes are very important in the cycling of elements, including carbon, nitrogen, sulfur and phosphorus, as well as metals and metalloids such as copper, mercury, selenium, arsenic and chromium. A full understanding of the complex biological phenomena that occur in the biosphere therefore requires a deep knowledge of the unique biological processes that occur in this vast prokaryotic world.

After publication in 1995 of the first full DNA sequence of a free-living bacterium, *Haemophilus influenzae*, whole genome sequences of hundreds of prokaryotes have now been determined and many others are currently being sequenced (www.genomesonline.org/). Our knowledge of the whole genome profoundly influences all aspects of microbiology. Determination of entire genome sequences, however, is only a first step in fully understanding the properties of an organism and the environment in which the organism lives. The functions encoded by these sequences need to be elucidated to give biochemical, physiological and ecological meaning to the information. Furthermore, sequence analysis indicates that the biological functions of substantial portions of complete genomes are so far unknown. Defining the role of each gene in the complex cellular metabolic network is a formidable task. In addition, genomes contain hundreds to thousands of genes, many of which encode multiple proteins that interact and function together as multicomponent systems for accomplishing specific cellular processes. The products of many genes are often co-regulated in complex signal transduction networks, and understanding how the genome functions as a whole presents an even greater challenge. It is also known that for a significant proportion of metabolic activities, no representative genes have been identified across all organisms, such activities being

termed 'orphan' to indicate they are not currently assigned to any gene. This also represents a major future challenge and will require both computational and experimental approaches.

It is widely accepted that less than 1% of prokaryotes have been cultivated in pure culture under laboratory conditions. Development of new sequencing techniques has allowed us to obtain genomic information from the multitudes of unculturable prokaryotic species and complex microbial populations that exist in nature. Such information might provide a basis for the development of new cultivation techniques. Elucidation of the function of unknown genes through a better understanding of biochemistry and physiology could ultimately result in a fuller understanding of the complex biological phenomena occurring in the biosphere.

Unlike multicellular eukaryotes, individual cells of unicellular prokaryotes are more exposed to the continuously changing environment, and have evolved unique structures to survive under such conditions. Chapter 2 describes the main aspects of the composition and structure of prokaryotic cells.

Life can be defined as a reproduction process using materials available from the environment according to the genetic information possessed by the organism. Utilization of the materials available in the environment necessitates transport into cells that are separated from the environment by a membrane. Chapter 3 outlines transport mechanisms, not only for intracellular entry of nutrients, but also for excretion of materials including extracellular enzymes and materials that form cell surface structures.

Many prokaryotes, including *Escherichia coli*, can grow in a simple mineral salts medium containing glucose as the sole organic compound. Glucose is metabolized through glycolytic pathways and the tricarboxylic acid (TCA) cycle, supplying all carbon skeletons, energy in the form of ATP and reducing equivalents in the form of NADPH for growth and reproduction. Glycolysis is described in Chapter 4 with emphasis on the reverse reactions of the EMP pathway and on prokaryote-specific metabolic pathways. When substrates other than glucose are used, parts of the metabolic pathways are employed in either forward or reverse directions. Chapter 5 describes the TCA cycle and related metabolic pathways, and energy transduction mechanisms. Chapter 6 describes the biosynthetic metabolic processes that utilize carbon skeletons, ATP and NADPH, the production of which is discussed in the previous chapters. These chapters summarize the biochemistry of central metabolism that is employed by prokaryotes to enable growth on a glucose–mineral salts medium.

The next five chapters describe metabolism in some of the various trophic variations found in prokaryotes. These are the use of organic compounds other than glucose as carbon and energy sources (Chapter 7), anaerobic fermentation (Chapter 8), anaerobic respiratory processes (Chapter 9), chemolithotrophy (Chapter 10) and photosynthesis (Chapter 11). Some of these metabolic processes are

prokaryote specific, while others are found in both prokaryotes and eukaryotes.

Prokaryotes only express a proportion of their genes at any given time, just like eukaryotes. This enables them to grow in the most efficient way under any given conditions. Metabolism is regulated not only through control of gene expression but also by controlling the activity of enzymes. These regulatory mechanisms are discussed in Chapter 12. Finally, the survival of prokaryotic organisms under starvation conditions is discussed in terms of storage materials and resting cell structures in Chapter 13.

This book has been written as a text for senior students at undergraduate level and postgraduates in microbiology and related subjects. A major proportion of the book has been based on review papers published in various scientific journals including those listed below:

Annual Review of Microbiology
Annual Review of Biochemistry
Current Opinion in Microbiology
FEMS Microbiology Reviews
Journal of Bacteriology
Microbiology and Molecular Biology Reviews (formerly *Microbiology Reviews*)
Nature Reviews Microbiology
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2

Composition and structure of prokaryotic cells

Like all organisms, microorganisms grow, metabolize and replicate utilizing materials available from the environment. Such materials include those chemical elements required for structural aspects of cellular composition and metabolic activities such as enzyme regulation and redox processes. To understand bacterial metabolism, it is therefore helpful to know the chemical composition of the cell and component structures. This chapter describes the elemental composition and structure of prokaryotic cells, and the kinds of nutrients needed for biosynthesis and energy-yielding metabolism.

2.1 Elemental composition

From over 100 natural elements, microbial cells generally only contain 12 in significant quantities. These are known as major elements, and are listed in Table 2.1 together with some of their major functions and predominant chemical forms used by microorganisms.

They include elements such as carbon (C), oxygen (O) and hydrogen (H) constituting organic compounds like carbohydrates. Nitrogen (N) is found in microbial cells in proteins, nucleic acids and coenzymes. Sulfur (S) is needed for S-containing amino acids such as methionine and cysteine and for various coenzymes. Phosphorus (P) is present in nucleic acids, phospholipids, teichoic acid and nucleotides including NAD(P) and ATP. Potassium is the major inorganic cation (K^+), while chloride (Cl^-) is the major inorganic anion. K^+ is required as a cofactor for certain enzymes, e.g. pyruvate kinase. Chloride is involved in the energy conservation process operated by halophilic archaea (Section 11.6). Sodium (Na^+) participates in several transport and energy transduction processes, and plays a crucial role in microbial growth under alkaline conditions (Section 5.7.4). Magnesium (Mg^{2+}) forms complexes with phosphate groups including those found in nucleic acids, ATP, phospholipids and lipopolysaccharides. Several microbial intracellular enzymes, e.g. monomeric alkaline phosphatase, are calcium dependent. Ferrous and ferric ions play a

Table 2.1. *Major elements found in microbial cells with their functions and predominant chemical forms used by microorganisms*

Element	Atomic number	Chemical forms used by microbes	Function
C	6	organic compounds, CO, CO_2	major constituents of cell material in proteins, nucleic acids, lipids, carbohydrates and others
O	8	organic compounds, CO_2, H_2O, O_2	
H	1	organic compounds, H_2O, H_2	
N	6	organic compounds, NH_4^+, NO_3^-, N_2	
S	16	organic sulfur compounds, SO_4^{2-}, HS^-, S^0, $S_2O_3^{2-}$	proteins, coenzymes
P	15	HPO_4^{2-}	nucleic acids, phospholipids, teichoic acid, coenzymes
K	19	K^+	major inorganic cation, compatible solute, enzyme cofactor
Mg	12	Mg^{2+}	enzyme cofactor, bound to cell wall, membrane and phosphate esters including nucleic acids and ATP
Ca	20	Ca^{2+}	enzyme cofactor, bound to cell wall
Fe	26	Fe^{2+}, Fe^{3+}	cytochromes, ferredoxin, Fe-S proteins, enzyme cofactor
Na	11	Na^+	involved in transport and energy transduction
Cl	17	Cl^-	major inorganic anion

crucial role in oxidation–reduction reactions as components of electron carriers such as Fe-S proteins and cytochromes.

In addition to these 12 major elements, others are also found in microbial cells as minor elements (Table 2.2). All the metals listed in Table 2.2 are required for specific enzymes. It is interesting to note that the atomic number of tungsten is far higher than that of the other elements and that this element is only required in rare cases.

2.2 Importance of chemical form

2.2.1 Five major elements

The elements listed in Tables 2.1 and 2.2 need to be supplied or be present in the chemical forms that the organisms can use. Carbon is the most abundant element in all living organisms. Prokaryotes are broadly classified according to the carbon sources they use: organotrophs (heterotrophs) use organic compounds as their carbon source while CO_2 is used by lithotrophs (autotrophs). These groups

Table 2.2. *Minor elements found in microbial cells with their functions and predominant chemical form used by microorganisms*

Element	Atomic number	Chemical form used by microbes	Function
Mn	23	Mn^{2+}	superoxide dismutase, photosystem II
Co	27	Co^{2+}	coenzyme B_{12}
Ni	28	Ni^{+}	hydrogenase, urease
Cu	29	Cu^{2+}	cytochrome oxidase, oxygenase
Zn	30	Zn^{2+}	alcohol dehydrogenase, aldolase, alkaline phosphatase, RNA and DNA polymerase, arsenate reductase
Se	34	SeO_3^{2-}	formate dehydrogenase, glycine reductase
Mo	42	MoO_4^{2-}	nitrogenase, nitrate reductase, formate dehydrogenase, arsenate reductase
W	74	WO_4^{2-}	formate dehydrogenase, aldehyde oxidoreductase

are divided further according to the form of energy they use: chemotrophs (chemoorganotrophs and chemolithotrophs) depend on chemical forms for energy while phototrophs (photoorganotrophs and photolithotrophs) utilize light energy ('organo' refers to an organic substance while 'litho' refers to an inorganic substance).

Nitrogen sources commonly used by microbes include organic nitrogenous compounds such as amino acids, and inorganic forms such as ammonium and nitrate. Gaseous N_2 can serve as a nitrogen source for a limited number of nitrogen-fixing prokaryotes. Nitrogen fixation is not known in eukaryotes. Some chemolithotrophs can use ammonium as their energy source (electron donor, Section 10.2) while nitrate can be used as an electron acceptor by denitrifiers (Section 9.1).

Sulfate is the most commonly used sulfur source, while other sulfur sources used include organic sulfur compounds, sulfide, elemental sulfur and thiosulfate. Sulfide and sulfur can serve as electron donors in certain chemolithotrophs (Section 10.3), and sulfate and elemental sulfur are used as electron acceptors and reduced to sulfide by sulfidogens (Section 9.3).

2.2.2 Oxygen

Oxygen in cells originates mainly from organic compounds, water or CO_2. Molecular oxygen (O_2) is seldom used in biosynthetic processes. Some prokaryotes use O_2 as the electron acceptor, but some cannot grow in its presence. Thus, organisms can be grouped according to their reaction with O_2 into aerobes that require O_2, facultative anaerobes that use O_2 when it is available but can also grow in its absence, and obligate anaerobes that do not use O_2. Some obligate anaerobes cannot grow and/or lose their viability in the presence of O_2 while others can tolerate it. The former are termed strict anaerobes and the latter aerotolerant anaerobes.

Table 2.3. *Common growth factors required by prokaryotes and their major function*

Growth factor	Function
p-aminobenzoate	part of tetrahydrofolate, a one-carbon unit carrier
Biotin	prosthetic group of carboxylase and mutase
Coenzyme M	methyl carrier in methanogenic archaea
Folate	part of tetrahydrofolate
Hemin	precursor of cytochromes and hemoproteins
Lipoate	prosthetic group of 2-keto acid decarboxylase
Nicotinate	precursor of pyridine nucleotides (NAD^+, $NADP^+$)
Pantothenate	precursor of coenzyme A and acyl carrier protein
Pyridoxine	precursor of pyridoxal phosphate
Riboflavin	precursor of flavins (FAD, FMN)
Thiamine	precursor of thiamine pyrophosphate
Vitamin B_{12}	precursor of coenzyme B_{12}
Vitamin K	precursor of menaquinone

2.2.3 Growth factors

Some organotrophs such as *Escherichia coli* can grow in simple media containing glucose and mineral salts, while others, like lactic acid bacteria, require complex media containing various vitamins, amino acids and nucleic acid bases. This is because the latter organisms cannot synthesize certain essential cellular materials from only glucose and mineral salts. These required compounds should therefore be supplied in the growth media: such compounds are known as growth factors. Growth factor requirements differ between organisms with vitamins being the most commonly required growth factors (Table 2.3).

2.3 Structure of microbial cells

Microorganisms are grouped into either prokaryotes or eukaryotes according to their cellular structure. With only a few exceptions, prokaryotic cells do not have subcellular organelles separated from the cytoplasm by phospholipid membranes such as the nuclear and mitochondrial membranes. Organelles like the nucleus, mitochondria and endoplasmic reticulum are only found in eukaryotic cells. The detailed structure of prokaryotic cells is described below.

2.3.1 Flagella and pili

Motile prokaryotic cells have an appendage called a flagellum (plural, flagella) involved in motility, and a similar but smaller structure, the fimbria (plural, fimbriae). Fimbriae are not involved in motility and are composed of proteins.

The bacterial flagellum consists of three parts. These are a basal body, a hook and a filament (Figure 2.1). The basal body is embedded in the cytoplasmic membrane and cell surface structure and