PROKARYOTIC GROWTH, NUTRITION AND PHYSIOLOGY

T. G. Downing,
Department of Biochemistry & Microbiology, University of Port Elizabeth, South Africa

Keywords: growth, physiology, nutrition, respiration, fermentation, photosynthesis.

Contents

1. Introduction

Prokaryotes possess an enormous diversity of morphological, nutritional, ecological and genetic features. This diversity is reflected in physiological and growth characteristics of different bacteria. In view of this enormous diversity, any discussion of prokaryotic growth and physiology must rely heavily on generalizations and specific examples to illustrate general themes. In this introduction to various aspects of bacterial physiology and growth, an energetic and nutritional approach will be used, since clearly the growth responses and physiological variations and adaptations are a function of immediate and long-term environmental conditions, which in turn define the physiology and growth of the bacteria.

Major changes in growth and physiology are observed as a function of nutrient availability (including light availability for phototrophs), temperature, oxygen or alternative electron acceptor availability, and any other specific growth requirements such as osmolarity and pH. In order to fully appreciate the impact of these external growth parameters, we need to have a basic understanding of bacterial growth and associated processes. What follows is an attempt to cover most of the major areas of bacterial growth, nutrition and physiology, and the variations and adaptations that occur across taxonomic divisions, temporally, and as a function of short-term environmental variations and stresses. In order to do this, much detail is relinquished in favor of scope.

2. Bacterial Cell Growth and Division

Cell growth, as distinguished from population growth, requires a coordinated, but not necessarily synchronized, increase in the amounts of cytoplasmic components, and cell membrane and cell wall material. The process of cellular growth cannot be viewed simply as the growth of the cell envelope, replication of the genetic material and an increase in the cytoplasmic components, since these are interdependent variables.

Cell shape and size, are maintained by the peptidoglycan layer. Extensive research on cellular growth of *Escherichia coli*, has suggested an exponential increase in cell mass and volume. The mass and volume of rapidly growing cells is larger than that of slowly growing cells, despite the shorter generation time. The relative rates of growth along the length and width of the cells are also determined by the generation time, and rates of change of the generation time. Under conditions resulting in constant growth rate, the diameter of the cell remains constant and growth is along the long axis of the cell. Insertion of new peptidoglycan units occurs diffusely prior to the onset of cell division. It should also be noted that peptidoglycan unit incorporation does not imply growth, as the peptidoglycan is a fairly dynamic structure with repair and replacement occurring more or less continuously. During cell division, insertion occurs preferentially at the site of septum formation. This contrasts with the addition of components to the outer layers, as is evidenced by the continued diffuse addition of the outer membrane glycoporin LamB, and lipoproteins. During cell constriction, growth of peptidoglycan occurs at the leading edge of the constriction. Outer membrane changes appear to be passive, following the peptidoglycan lead. Periplasmic murein synthesis reactions are catalyzed by peniciliin binding proteins (PBPs). In *E. coli* the high molecular weight PBPs are PBP1A, PBP1B, PBP2 and PBP3. 1A and B appear to be required for general peptidoglycan synthesis, PBP2 (the major growth PBP) for cell growth along the long axis, and PBP3 for division. All the high molecular weight PBPs exhibit both transglycolase and transpeptidase activities. The low molecular weight PBPs, 4, 5 and 6 all have carboxypeptidase activity, and PBP4 also has endopeptidase activity. There is evidence to suggest that peptidoglycan synthesis may follow changes in cytoplasmic membrane component synthesis and incorporation.

3. The bacterial cell cycle and its regulation

Bacterial cells divide by binary fission, ternary fission, multiple fission, fragmentation, budding, or the production of spores, baeocytes or some other disseminative structure. These processes are tightly controlled by the cell and depend on a variety of parameters including nutritional status, cell density and physical parameters. During population growth, cell division and chromosome replication must be coordinated. During low growth rates, complete duplication of the chromosome occurs once for each cell division event. In order for this to occur, initiation of replication must occur prior to completion of the previous round of replication. In this case, each daughter cell receives a copy of the chromosome on which replication has already commenced. This model of replication, the Helmstetter-Cooper model, allows for the explanation of the growth rate affecting the chromosome number. Control of the initiation process is poorly understood. The protein DnaA appears to be involved in initiation in that it is required to bind at the origin of replication before replication can occur. The degree of

methylation of the origin of replication is also important. Initiation, as previously mentioned, must occur in a coordinated manner with growth rate. It has been suggested that the concentration of an intracellular metabolite may play a role in initiation. Lower concentrations, associated with increased rates of cell division, would accordingly initiate chromosomal replication at a higher rate. Guanosine 5′ diphosphate 3′ diphosphate has been proposed as such a molecule, due to its growth rate dependant concentration and ability to inhibit DnaA synthesis at high concentrations.

Cellular morphogenesis is under the control of the so-called morphogenes, the products of which ensure coordinated cellular component production and the correct positioning of these products in the cell. For example, the FtsZ protein, involved in septum ring formation during cell division, must be positioned centrally in the cell. One model proposes the *minC* and *minD* gene products binding at polar regions thus preventing the binding of FtsZ. The proposed MinCD complex is in turn prevented from binding at the central region by the *minE* gene product which binds at a position adjacent to the binding site for FtsZ. Another morphogene is *envA*, the product of which is involved with septum splitting. Whether the control of cellular growth and division and chromosome replication are dependently or independently controlled is a subject of debate. Evidence for independent control includes the inhibition of certain growth events without interference with division events. However, although there is no evidence for direct dual control, control may be coupled, as evidenced by the presence of general regulatory systems such as the SOS system, one component of which also inhibits the initiation of septum formation. The argument appears to be one of degree, however, with interdependence probably being the rule for coordinated cell cycle regulation.

4. Bacterial Population Growth

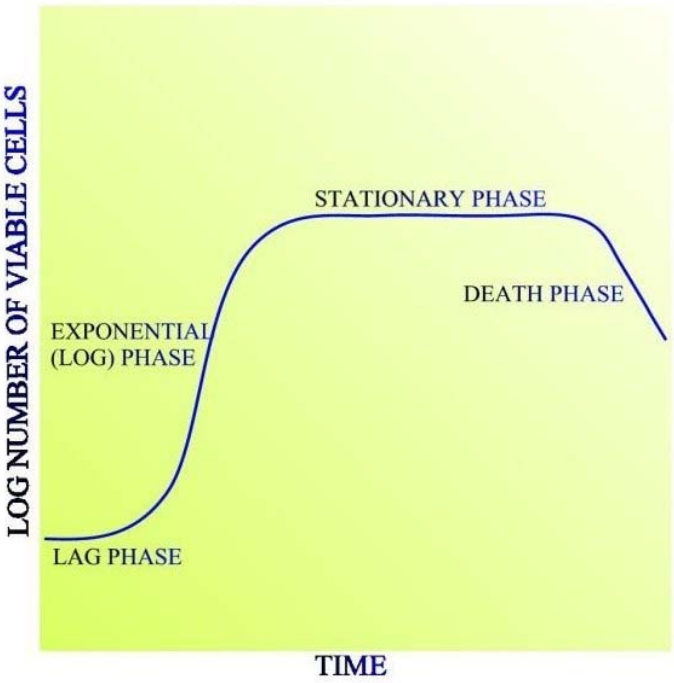

Figure 1. Typical growth curve obtained during batch culture of a bacterium.

The growth of bacteria that reproduce by binary fission is exponential. Growth in a closed system is characterized by the growth curve depicted in Figure 1.

The growth curve is typified by an initial lag phase during which cell division is not occurring as rapidly as is possible given the environmental conditions. This is due to the accumulation of sufficient nutrients and the coordination of growth events as a function of nutrient and other parameter values. During the logarithmic growth phase, the growth rate is constant and cell division occurs at given intervals for a particular cell and progeny. As the conditions change, this period (the generation time) gradually increases. This reduction in growth rate is typically due to nutrient depletion and/or waste product accumulation. This growth is usually described as follows for a single bacterium growing on a single limiting substrate:

$$\mu = \frac{\mu_{\max} . s}{K_S + s}$$

Where μ is the specific growth rate, μ_{max} is the maximal specific growth rate for the organism, s is the substrate concentration in the medium, and K_S is the saturation constant for uptake of the substrate by the bacterium. This applies equally to continuous culture systems where $\mu = D$ (D being the dilution rate for the culture vessel) and the critical dilution rate, D_c, is the dilution rate at which biomass washout occurs; therefore $D_c = \mu_{max}$ for the given conditions.

Clearly microbial population growth is substantially more complex than described here, and must take into account such factors as the effect of modulation of environmental parameters, alternative preferred or multiple substrates, toxic waste products etc. The mathematical description of microbial population growth is discussed in *Mathematical Biology* .

5. Bacterial Nutrition

The so-called macroelements, carbon, nitrogen, phosphorous, oxygen, sulfur and hydrogen are the major components of bacterial cells. The remaining macronutrients, potassium, calcium, iron and magnesium, although essential, comprise a relatively small percentage of the total cell mass. The trace elements such as cobalt, manganese, copper, molybdenum, nickel and zinc are also required by most cells, but in amounts that make it difficult to measure the actual requirements. In addition to the necessary ability to catabolise or assimilate and utilize molecules containing the necessary components, the bacterium must also be able to internalize such compounds. The variety of nutrient and energy sources used by bacteria is reflected in the variation in metabolism. The reader is referred to *Prokaryote Diversity* where some indication of this variation is discussed in the context of prokaryotic diversity. The following sections deal with the nutrient sources and mechanisms for internalization or assimilation of the major nutrients, and with methods of energy generation. Clearly these subjects are interdependent since much of the energy obtained by chemotrophs will be from assimilated inorganic or organic molecules, and in many cases energy is required for the assimilation and/or internalization of these molecules. Similarly for autotrophs, the interdependence of these processes is obvious in that phototrophic autotrophy not only provides energy, but

also allows for such processes as photophosphorylation and carbon fixation. It is hoped that the division into the following sections, and indeed the order of the sections, is such that each section naturally follows the preceding one, and such that they may stand independently.

5.1. Carbon sources and assimilation

Carbon typically constitutes about 50% of the dry cell mass, and may be obtained chemotrophically, or autotrophically. Chemotrophs may be chemoorganotrophic or chemolithotrophic, obtaining their carbon from pre-reduced organic molecules or inorganic molecules respectively. Autotrophs fix CO_2 by photosynthesis. Organic molecules such as sugars, amino acids and lipids must be taken up by the cell to be catabolised. Uptake systems for these molecules are essential since the cytoplasmic membrane is an efficient barrier to large and charged molecules. A variety of transport systems, typically specific for the substrate molecule, exist. Often more than one transport system may be present for a particular substrate. Many of the transport systems are energy dependant, and use proton motive force or ATP for the uptake of the nutrient. In *Escherichia coli* the proton-lactose system is an example of the former, with proton motive force being reduced in exchange for lactose, which is transported into the cytoplasm together with protons. The use of ATP to provide the necessary energy for uptake of molecules is common, and is typified by ABC transporter systems, so named because of the presence of an ATP binding cassette in the multi-protein complex. ABC type importers mediate the uptake of certain amino acids, sugars and ions. ABC exporters are involved in the export of various polypeptides including enzymes and antibiotics, and in the export of polysaccharide capsule components in some species. Proteins secreted by these systems typically lack the N-terminal signal sequence associated with export by the general secretory pathway of Gram negative bacteria, but do have a consensus C-terminal sequence thought to associate with an ABC protein.

The phosphotransferase system (PTS) is the best studied of the group translocation systems. This system is widely distributed amongst the prokaryotes, and is present in many facultatively anaerobic bacteria such as *Escherichia, Salmonella,* and *Staphylococcus*, but is not found in aerobes, with the exception of bacteria possessing both the Embden-Meyerhof and phosphotransferase systems, such as *Bacillus*. Some obligate anaerobes such as *Clostridium* also have the PTS. The PTS in *Escherichia* and *Salmonella* consists of a cytoplasmic membrane-embedded enzyme, EII, often consisting of three domains (EIIA, EIIB and EIIC). EII is however variable in structure, with one of the domains (EIIA) being dissociated and cytoplasmic. Although hydrophilic, EIIB is often attached to the hydrophobic, membrane domain, EIIC. In addition to the EII enzyme, another enzyme EI and a low molecular weight heat stable protein, HPr, are also present. These three components transfer a phosphate from phosphoenolpyruvate to the sugar molecule as it is transported across the membrane by EII. Although HPr and EI are common to all PTS systems, EII is specific for particular sugars. Figure 2 shows a diagrammatic representation of the PTS of *Escherichia*.

Most chemolithotrophic organisms are autotrophic, using CO_2 as their carbon source, although mixotrophy is not uncommon. Photoautotrophy is likewise more common than photoheterotrophy. The fixation of CO_2 may occur via several biochemical pathways.

The most widespread of these systems is the Calvin cycle, which requires both NAD(P)H and ATP, and uses the enzymes ribulose bisphosphate carboxylase (RubisCO) and phosphoribulokinase. RubisCO is widely distributed amongst the prokaryotes, and is found in the cyanobacteria, purple bacteria and most chemolithotrophic bacteria, as well as some Archaea.

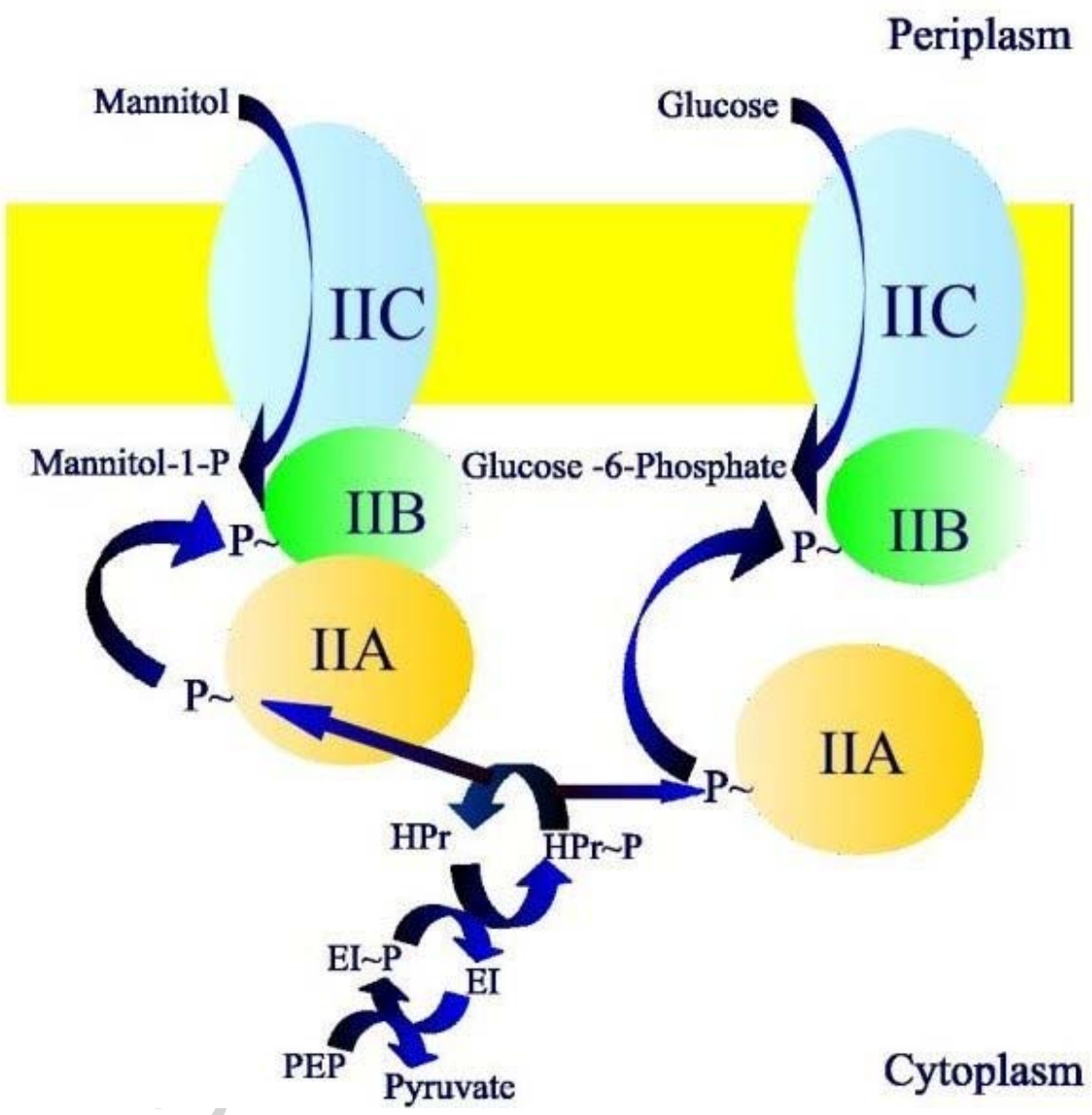

Figure 2. Diagrammatic representation of the PTS of *Escherichia*.

RubisCO catalyzes the formation of two molecules of phosphoglyceric acid from one molecule of ribulose bisphosphate and one CO_2 molecule. The phosphoglyceric acid is then phosphorylated by ATP and reduced to form glyceraldehyde-3-phosphate. Ribulose bisphosphate is produced by phosphorylation of ribulose-5-phosphate by ATP. This reaction is catalyzed by phosphoribulokinase. The overall stoichiometry of the Calvin cycle is as follows:

$$6\ CO_2 + 12\ NADPH + 18\ ATP \rightarrow C_6H_{12}O_6(PO_3H_2) + 12\ NADP^+ + 18\ ADP + 17\ P_i$$

The reader is referred to section *Carbon Fixation-C3 and C4 Pathways* for details of the biochemistry of this cycle.

Strict autotrophs which use RubisCO, have cytoplasmic inclusions called carboxysomes that are about 100 nm in diameter and are surrounded by a non-unit membrane. These inclusions contain a crystalline array of RubisCO molecules. It is thought that these may be an evolutionary adaptation to allow for increased RubisCO activity without affecting the osmolarity of the cytoplasm, since the carboxysomes are insoluble.

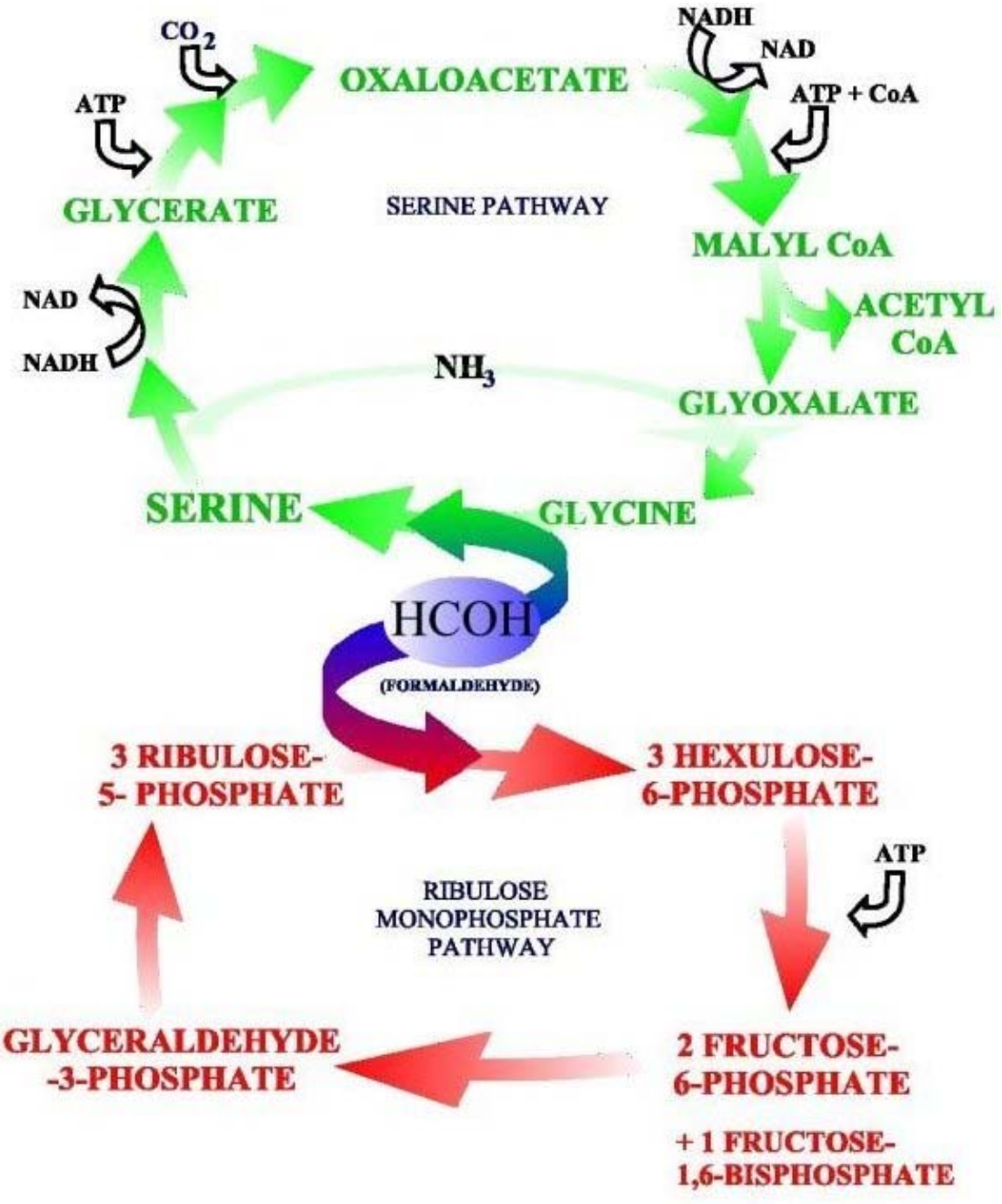

Figure 3. Pathways for assimilation of C_1 derived formaldehyde carbon: (a) the ribulose monophosphate pathway and (b) the serine pathway.

Green sulfur and green non-sulfur bacteria have alternative methods for carbon fixation. The green sulfur bacterium, *Chlorobium*, uses a reversal of certain steps of the citric acid cycle for CO_2 fixation. The citric acid cycle enzymes functions in reverse with the exception of citrate synthase, which is replaced by citrate lyase. Two unique ferrodoxin-linked enzymes catalyze the carboxylation of succinyl-CoA to α-ketoglutarate, and the carboxylation of acetyl-CoA to pyruvate. The reader is referred to *Carbon Fixation-C3 and C4 Pathways* for details of the biochemistry of this cycle. *Chloroflexus*, a green non-sulfur bacterium, contains a unique autotrophic pathway, the hydroxypropionate pathway. Acetyl-CoA is carboxylated twice to yield methylmalonyl-CoA which is rearranged to yield acetyl-CoA and glyoxylate.

In addition to autotrophy, methylotrophy and methanotrophy are other nutritional mechanisms for carbon assimilation of C_1 compounds. Methanotrophs—those methylotrophs capable of oxidizing methane—incorporate oxygen from O_2 into methane using the enzyme methane monooxygenase. Depending on the organism, one half or all of the resulting formaldehyde is assimilated as carbon. In *Methylosinus*, the most studied of the methylotrophs, the electrons for the oxidation of CH_4 to CH_3OH are obtained from cytochrome *c*. All further oxidations of methanol to CO_2 yield electrons which pass down the electron transport chain. Carbon from the formaldehyde is assimilated either via the ribulose monophosphate pathway or via the serine pathway. The ribulose monophosphate pathway for C_1 assimilation by type I methanotrophs such as *Methylobacter*, *Methylococcus* and *Methylomonas* is the more efficient one since all carbon atoms are derived from formaldehyde and requires no reducing power. One ATP is used for each glyceraldehyde-3-phosphate produced. Figure 3 depicts the ribulose monophosphate pathway and the serine pathway. In the serine pathway, one molecule of acetyl-CoA is synthesized from one molecule of formaldehyde and one CO_2. The reactions require two molecules of NADH and two ATPs for each acetyl-COA synthesized. In addition to several citric acid cycle enzymes, one unique enzyme, serine transhydroxymethylase is used.

-
-
-

Bibliography

Balows A, Truper HG, Dworkin M, Harder W & Schleifer, K-H (1992). *The Prokaryotes*, 2d Ed, Springer-Verlag, New York.

Caldwell DR (2000). *Microbial Physiology and Metabolism*, 2nd ed. Star Publishing, Belmont, California.

Dawes EA (1986). *Microbial Energetics*. Chapman, New York.

Ingledew WJ & Poole RK (1984). *The Respiratory Chains of* Escherichia coli. Microbiol. Rev **48(3):**222-271.

Moat AG & Foster JW (1995). *Microbial Physiology*, 3rd ed. John Wiley & Sons, Inc, Yew York.

Shlegel HG & Bowien B (eds) (1989). *Autotrophic Bacteria* Scince Tech Publishers, Madison, Wisconsin.

Singleton P (1997). *Bacteria in Biology, Biotechnology and Medicine*, John Wiley & Sons New York.

Smith AJ & Hoare DS (1977). *Specialist phototrophs, lithotrophs and methylotrophs: A unity among diversity of prokaryotes?* Bacteriol. Rev. **41(1):**419-448.

White D (1995). *The physiology and Biochemistry of Prokaryotes*. Oxford University Press, Yew York.