Chapter 2

Structure and Growth of Microbial Populations

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1. INTRODUCTION

This work, in part, documents how prokaryotes play an environmentally significant to dominant role in the cycling of numerous important redox-sensitive elements such as carbon, iron, manganese, oxygen, nitrogen, and sulfur. This Chapter explores some of the principles outlining the structure and growth of the populations conducting these transformations. We consider the factors regulating population growth and population size. We explore how populations with overall similar physiology can occupy an enormous range of environmental conditions, including extremes of temperature, salinity, and pH. We also look at factors influencing the ecology of microbial populations. Indeed, we know relatively little about the details of microbial interactions in nature, but we will overview what we do understand. In

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addition, we explore how cells communicate with each other and how, at least in some circumstances, they cooperate to effectively utilize available resources. The recognition that prokaryotes can display community behavior is an important revelation in microbial ecology (e.g., Shapiro, 1998; Miller and Bassler, 2001).

2. CONSIDERATIONS OF CELL SIZE

It is no secret that prokaryotes are small, with typical nominal cell diameters of between 0.5 μm and 2 μm. In fact, smallness has decided advantages, as prokaryotes obtain their nutritional requirements from the environment by diffusion. Other modes of transport such as advection or dispersion operate on spatial scales too large to influence transport of nutrients to microbes (Fenchel *et al.*, 1998). Indeed, over small-distance scales, diffusion is a remarkably effective transport process, whose timescale can be estimated from the Stokes-Einstein relationship. Here, the timescale (t) associated with transport over a characteristic length scale (L) depends on the length scale and the diffusion coefficient (D) (Equation 2.1):

$$t = \frac{L^2}{2D} \tag{2.1}$$

If we take $D = 1 \times 10^{-5}$ cm^2 s^{-1}, which is not uncommon for gases in solution, then values for t may be calculated for a range of characteristic-length scales, L, as shown in Table 2.1. It is apparent that diffusion is extremely rapid, with timescales of approximately 1 ms, over the length scales appropriate for transport to small prokaryotic organisms. Thus, small size allows rapid transport of nutrients to an actively growing cell.

Still, we may assume that even small cells can experience diffusion-limited growth in nature, particularly if the concentration of the limiting substrate is

Table 2.1 Time associated with diffusion over various characteristic-length scales

Length	Time
1 μm	0.5 ms
10 μm	50 ms
100 μm	5 s
1 mm	8 min
1 cm	14 hr
10 cm	58 d

low. The total diffusional flux of substrate to the surface of a spherical cell is given by

$$J = 4\pi Dr(C_\infty - C_o) \tag{2.2}$$

where r is the cell radius, D is the diffusion coefficient, C_∞ is the concentration of the substrate in the bulk reservoir and C_o is the concentration of the substrate at the cell surface (Fenchel *et al.*, 1998; Schulz and Jørgensen, 2001). The maximum diffusional flux occurs when the concentration of the limiting substrate is zero at the cell surface ($C_o = 0$):

$$J_{\max} = 4\pi DrC_\infty \tag{2.3}$$

We can now calculate the volume-specific rates of metabolism under diffusion limitation by dividing Equation 2.3 with the volume of the cell ($V = 4/3\pi r^3$), to yield the following:

$$\text{Specific rate} = 3DC_\infty/r^2 \tag{2.4}$$

Therefore, under diffusion limitation, the specific rate of cell metabolism should increase linearly with substrate concentration (C_∞) and decrease with the square of the cell radius (r^2). This is a strong indication that small cell size is a decided advantage in maintaining high specific rates of metabolism.

Yet, there is a limit to how small cells can be. This issue was brought into sharp focus by the report of McKay *et al.* (1996) of 20 nm to 100 nm objects from the Martian meteorite AHL84001, which they believed to be fossil cells. Theoretical considerations suggest that a minimum cell size should fall in the range of 170 nm. This value is obtained assuming a cell genome housing 250 genes (~300 kilobases), which is considered the minimal number needed to support heterotrophic cell growing in a nutrient-rich environment, in other words, a cell with minimal metabolic complexity (Adams, 1998). Furthermore, it is assumed that the cell contains 10% ribosomes, 20% proteins, and 50% water, by analogy with typical prokaryote cells (Adams, 1998). In this case, the cell contains 65 ribosomes and 65 proteins per gene. Further calculations reveal that a cell size of just 50 nm, with a 5 nm cell wall, can accommodate only two ribosomes, 520 protein molecules, and a DNA strand containing only about eight genes. This is a biosynthetic factory too small to function in a manner we understand (Adams, 1998).

We can compare these calculations with the smallest known prokaryotes in nature, often referred to as nanobacteria. The recently discovered archaeon *Nanoarchaem equitans* is coccoidal with an extremely small cell diameter of approximately 400 nm (Huber *et al.*, 2002). This organism has the smallest known genome among prokaryotes at 500 kilobases, and it lives in intimate association with an autotrophic sulfur-reducing archaeon

of the genus *Ignicoccus*. *N. equitans* apparently represents the first member of a whole new kingdom of organisms within the *Archaea*. Other small *Archaea* are found among the genera *Thermofilum*, with rod diameters of 170 nm, *Thermoproteus,* with 300-nm-diameter elongated cells, and *Pyrobaculum,* with disk diameters of 200 to 300 nm and widths of 80 to 100 nm (Stetter, 1998). The pathogenic bacteria *Mycoplasma pneumoniae* has a cell diameter of only 200 nm, and very small bacteria have been discovered in human and cow blood, with typical diameters of 200 nm, or even smaller (Kajander and Çifçioglu, 1998). Indeed, some of these blood-associated nanobacteria pass through 0.1 μm filters (Kajander *et al.*, 1997), and transmission electron microscopy (TEM) observations show tiny cell forms as small as 50 nm in diameter (Kajander *et al.*, 1997). Therefore, numerous different small cells in nature approach the theoretical lower limit of 170 nm diameter, and some even seem to fall below this limit. However, it is an open question as to how cells function at diameters below 100 nm.

Despite the advantages of small size in allowing efficient nourishment of the cell, most prokaryotes grow larger than our theoretical lower cell-size limit. With a typical cell diameter of 1 to 2 μm, prokaryotes can accommodate relatively large genomes (2 to 4 megabases is common), allowing metabolic plasticity, which can be an advantage in chemically dynamic environments, as often found in nature. Therefore, metabolic flexibility is balanced against the efficiency of nutrient uptake. But what about prokaryotes growing to an extremely large size? The current world record holder for large size is the sulfide oxidizer *Thiomargarita namibiensis,* with cell diameters up to 750 μm, large enough to be seen by the naked eye (Schulz *et al.*, 1999). Most of the cell volume accommodates a large central vacuole (Figure 2.1), and active cell cytoplasm, concentrated at the external margins of the cell, comprises only 2% of the cell volume (Schulz *et al.*, 1999). The largest known heterotroph is *Epulopiscium fishelsoni*, found in the guts of tropical fish, with a maximum diameter of about 80 μm and a maximum length of 600 μm (Angert *et al.*, 1993).

Other large bacteria include the sulfide oxidizer *Thioploca araucae*, consisting of numerous cells of 30 to 40 μm in diameter making up filaments (also called trichomes) of up to 7 cm or more in length (Schulz *et al.*, 1996; see Chapter 9). Up to 100 individual filaments occupy a common sheath vertically oriented in the sediment. Some unsheathed filaments of *Beggiatoa*, also a sulfide oxidizer, are found in hydrothermal areas with diameters of up to 122 μm (Jannasch *et al.*, 1989). Similar to *Thiomargarita*, most of the volume of these large *Thioploca* (Figure 2.1) and *Beggiatoa* cells accommodates a central vacuole. Large size is also relatively common among filamentous cyanobacteria, with individual trichomes reaching diameters of 40 μm or more, as for example in some species of *Oscillatoria* (Schulz and Jørgensen, 2001).

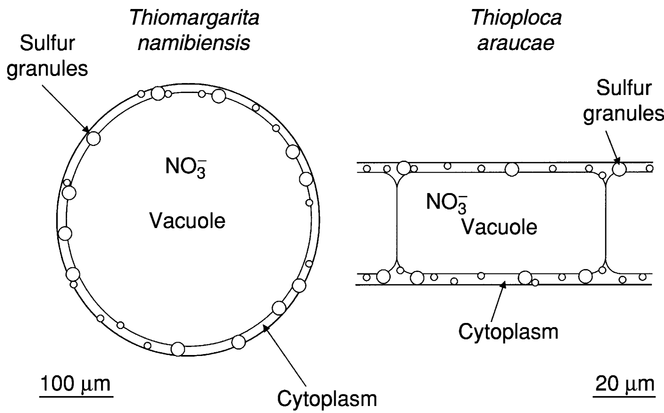

Figure 2.1 Relationships between the internal NO_3^--containing vacuoles and cell cytoplasm for *Thiomargarita namibiensis* and *Thioploca araucae.* Inspired from figures in Schulz *et al.* (1999) and Fossing *et al.* (1995).

Obviously, large size among these various organisms incurs some ecological advantage. Large vacuolated sulfide oxidizers concentrate nitrate into the vacuole for use as an electron acceptor in sulfide oxidation. With the cell cytoplasm concentrated in a thin layer near the cell membrane, the ratio between the volume of cytoplasm to the volume of vacuole will change with cell size in a manner similar to the surface area to volume ratio (surface area/volume = $3/r$). Therefore, as cell size increases, there is a greater volume of vacuole compared to the volume of cytoplasm and a greater supply of nitrate per volume of cytoplasm. This could be particularly important for an organism such as *T. namibiensis,* which is not motile and probably fills its "nitrate tank" during periodic resuspension events (Schulz *et al.*, 1999). It then relies on this nitrate supply to oxidize sulfide in sulfide-rich sediments that are normally devoid of nitrate (Schulz *et al.*, 1999).

For *Thioploca*, the nitrate tank is filled as *Thioploca* filaments extend out of their sheath into nitrate-rich water overlying the sediment surface. Filaments then migrate down the sheath into the sulfide-containing sediment below (Jørgensen and Gallardo, 1999). Therefore, the large cell size of *Thioploca* allows the accumulation of a large storage reservoir of nitrate, while the long filaments and common sheath allow a unique strategy for shuttling the electron acceptor (nitrate) to the electron donor (sulfide). For cyanobacteria, large size and filamentous structure allow the construction of resilient microbial mats that are not easily broken apart in turbulent environments.

3. POPULATION GROWTH

3.1. Substrate uptake and growth

Like all life on Earth, prokaryote populations must grow to be successful. The initial stages of growth in the laboratory are often very slow, and in some cases, no growth is apparent. This is known as the "lag phase." During this phase, the organisms are adapting to the culture conditions. This could mean repair of damaged cells if the culture is old or the synthesis of new enzymes if the culture conditions are different from what the organisms have previously seen. A lag phase, however, is not always observed. As an example, a freshly grown culture of *Desulfovibrio desulfuricans* begins growth immediately after inoculation into fresh media (Figure 2.2). After the lag phase, populations normally experience a period of exponential growth. The basis behind exponential growth is a doubling of population size during every cycle of cell division. If the frequency of cell division remains constant, as occurs with a rich supply of nutrients, and in the absence of inhibiting substances, then population growth is exponential with

$$N = N_o e^{\mu t} \tag{2.5}$$

where N (cells ml^{-1}) is population size at time t, N_o is the starting population size, and μ (h^{-1}) is the specific growth rate, which is related to the population doubling time t_D, defined as $t_D = \ln 2/\mu$. A stationary phase follows (Figure 2.2), where batch cultures typically reach cell densities of 10^8 to 10^{10} cells ml^{-1}, after which growth apparently stops. In this phase of growth,

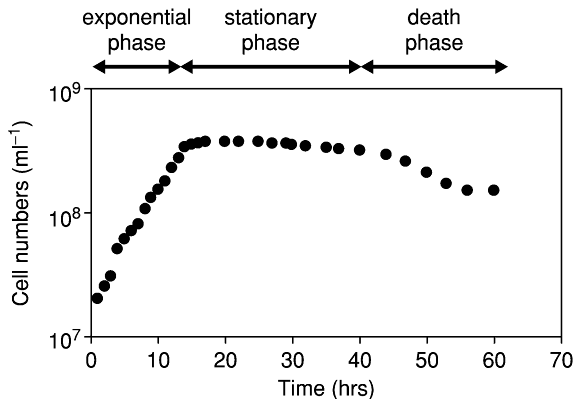

Figure 2.2 The stages of growth for a batch culture of the sulfate reducer *Desulfovibrio desulfuricans*. Results from Olesen and Canfield (unpublished).

cultures continue to metabolize substrate, but cell growth is nearly balanced by cell death. In this state a larger proportion of the energy gained during cellular metabolism is shuttled into cell function, with less used to support cell division. The stationary phase of growth begins after a vital nutrient or substrate becomes limiting in the culture media or when a harmful waste product accumulates to inhibitory levels. When cell growth cannot match cell death, the culture enters the "death phase" (Figure 2.2). Some cells may still multiply in this phase, but the medium may be too nutritionally poor, or too toxic, to support net growth.

Under all circumstances, there is a certain amount of energy used to support maintenance functions of the organism, including ongoing repair of cellular constituents, and the maintenance of chemical and electrical gradients needed to support cellular function (Pirt, 1975). Maintenance energy is a necessary expenditure by the organism, but it does not support growth. Thus, the energy derived from the metabolism of organisms under nutrient-limiting conditions is largely going toward maintenance functions, whereas when nutrients are abundant, energy is largely used for growth, although maintenance is still required. Maintenance energy, m_E, can be defined as the amount of energy dissipated per mol-equivalent of carbon substrate per time (kJ $Cmol^{-1}$ h^{-1}), and Tijhuis *et al.* (1993) have suggested, from a theoretical analysis, that m_E depends only on temperature. However, in the experiments of Scholten and Conrad (2000), maintenance energies were found to differ considerably from the theoretical values, suggesting that growth conditions other than temperature also influenced m_E.

The growth rates of prokaryote populations should logically depend, in some way, on the concentrations of critical substrates. Low concentrations should impose diffusion limitations on the cellular uptake of substrate, which limits growth rate. Also, if substrate concentration is too high, substrate transport sites at the cell surface become saturated, and increasing substrate concentration has no effect on substrate uptake rates. As part of his Ph.D. thesis, Jacques Lucien Monod (Monod, 1942) studied the relationship between growth rate and substrate concentration for strains of *Escherichia coli*. He found that the relationship could be described empirically through the now famous "Monod" equation:

$$\mu = \mu_{max} \frac{S}{K_S + S} \tag{2.6}$$

where μ (h^{-1}) is the specific growth rate, μ_{max} (h^{-1}) is the maximum specific growth rate, S (μM) is substrate concentration, and K_s (μM) is the half saturation constant, or the substrate concentration at half maximum growth rate. The magnitude of K_s indicates the affinity of the organism for a given substrate, with a lower K_s value meaning a higher substrate affinity. The

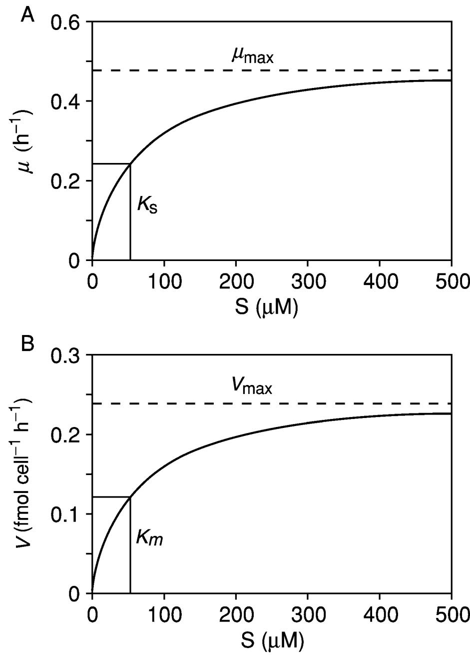

Figure 2.3 The relationship between μ_{max}, S, K_s and μ for microbial growth as given by the Monod equation (Equation 2.6) and between V_{max}, S, K_m and V for substrate utilization rate as given by the Michaelis-Menten equation (Equation 2.7).

relationship between these parameters is shown in Figure 2.3A. While the Monod equation was empirically derived, several theoretically based growth models produce a similar relationship (Panikov, 1995).

Microorganisms frequently also show similar relationships between substrate concentration and metabolic rate:

$$V = V_{max} \frac{S}{K_m + S} \tag{2.7}$$

where V is specific metabolic rate (μmol $g_{organisms}^{-1}$ h^{-1}, or fmol cell^{-1} h^{-1}), V_{max} is maximum metabolic rate, S is substrate concentration (μM), and K_m is the half saturation constant, or the substrate concentration at half maximum rate. The kinetic form shown in Equation 2.7 (Figure 2.3B) is often referred to as "Michaelis-Menten" kinetics, as Michaelis and Menten first derived a similar relationship from theoretical grounds for pure enzyme reactions (Michaelis and Menten, 1913). If two substrates are critical for metabolism, such as oxygen (electron acceptor) and ammonium (electron donor) for nitrifiers, the dual influence of substrate limitation on

metabolic rate can often be approximated by the following modification of the Michaelis-Menten expression:

$$V = V_{max}\left[\frac{S_D}{K_{mD} + S_D}\right]\left[\frac{S_A}{K_{mA} + S_A}\right] \tag{2.8}$$

where the subscripts D and A refer to electron donor and electron acceptor, respectively.

The kinetic parameters describing the growth and metabolism of individual organisms are somewhat variable depending on the previous history of the organism, the extent of substrate limitation, and the timescale of the observation (Button, 1985). For example, organisms can adapt to substrate limitation by increasing the density of transporter enzymes, which would increase V_{max}. Generally, but not always, high values of K_m are correlated with high V_{max}, while low values of K_m are correlated with low V_{max}. These different adaptations to substrate availability allow organisms to exploit different ecological niches in a dynamic environment. Thus, organisms with high K_m and V_{max} can metabolize (and grow) rapidly with a sudden input of fresh substrate, while organisms with low K_m and V_{max} are adapted to situations of substrate limitation.

This general relationship between K_m and V_{max} can be rationalized as follows. Organisms with low K_m have transporter or metabolic enzymes with high substrate affinity, which also tend to bind the substrate tightly with a relatively long lifetime for the substrate–enzyme complex. This relatively long lifetime for the intermediate complex decreases V_{max} (Button, 1985). Conversely, organisms with a lower affinity for substrate have transporter or metabolic enzymes binding substrate less tightly. This leads to a higher K_m, but also to a higher V_{max} due to a shorter lifetime for the substrate–enzyme complex. The relationship between K_m and V_{max} for sulfate uptake by sulfate reducers is shown in Figure 2.4 (data from Widdel, 1988).

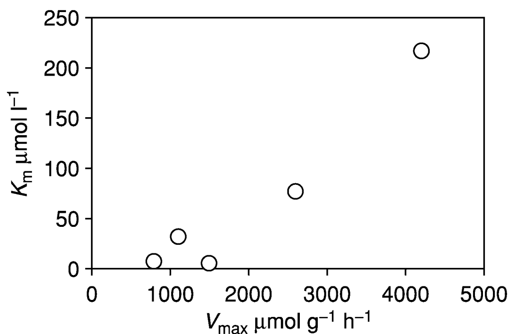

Figure 2.4 The relationship between K_m and V_{max} for acetate-utilizing sulfate reducers. Data from Widdel (1988).

The specific affinity, a_A (Equation 2.9), has been advocated as a fundamental parameter describing the affinity of prokaryotes for substrate (e.g., Button, 1986):

$$a_A = \frac{\mu_{max}}{K_s} \tag{2.9}$$

Specific affinity approximates the initial slope of a growth (μ) vs. substrate concentration (K) plot and provides an indication of how an organism's growth rate will respond to low substrate concentrations as generally found in nature. Hence, this parameter has ecological significance. Obviously, high specific affinities are advantageous for organisms growing under low substrate conditions. Nedwell (1999) found that within populations of bacteria and algae, specific affinities were heavily influenced by temperature. Thus, as temperature was reduced, specific affinity decreased, reflecting a stiffening of the cytoplasmic membrane, reducing the efficiency of transporter enzymes and proteins. We will discuss more of the relationship between temperature and metabolism below.

3.2. Growth yield

Growth yield, Y, expresses the amount of cell material formed during the utilization of a certain amount of substrate. This relationship is usually expressed in terms of mass, where

$$Y = \frac{\text{grams of dry cells formed}}{\text{grams of substrate consumed}} \tag{2.10}$$

A wide variety of different substrates can be chosen, including electron donor, electron acceptor or even a limiting nutrient. The definition of growth yield can also be modified to ratio cell formation against typical metabolic products, including, for example, ATP, O_2 or H_2. Growth yields are usually constant when organisms are in exponential growth (Fuchs and Kröger, 1999). Growth yield also tends to correlate with specific growth rate (μ), such that higher specific growth rates generate higher growth yields (up to a theoretical maximum, of course). In batch culture or chemostat experiments, high specific growth rates occur at relatively low population density where conditions are most favorable for growth (see above). At high population densities in batch cultures substrates become limiting and toxic waste products accumulate, slowing the rates of specific growth (see above) and reducing the growth yield. In this situation, a higher proportion of the energy gained during substrate utilization is channeled into maintenance rather than growth. A positive relationship between growth yield and specific growth rate probably also holds in nature.

Continuous culture

A continuous culture, or chemostat, provides a valuable means for exploring the growth physiology of microorganisms. A chemostat is a flow-through system in which growth substrates are introduced at a constant rate, allowing growth of a microbial culture. The volume is constant, so solution exits at the same rate as it is introduced, and in the output, microorganisms are removed at the same density as found within the chemostat. Therefore, in a chemostat, growth rates and population density are in a dynamic balance between rates of substrate input and rates of microorganism output. Microorganisms experience different growth conditions as input substrate concentrations and their rates of addition to the chemostat are manipulated. As substrates are usually consumed to low concentrations, input substrate concentration controls population density and substrate input rate controls growth rate. In a chemostat there is usually control over temperature, pH and oxygen levels when appropriate. In principle, chemostats develop to a steady state, with constant solution chemistry, microbial growth rates, and population size. This allows the exploration of growth physiology for organisms under constant conditions.

Population growth in a chemostat is assumed to follow Monod kinetics (Equation 2.6). The dilution rate, or residence time, of medium in the chemostat, D (time^{-1}), is equivalent to F/V, where F is the flow rate of medium into the chemostat (volume time^{-1}) and V is the volume. The growth rate of organisms is given by μX, where μ is the specific growth rate and X is population density (usually, grams dry weight liter^{-1}), and the loss or output rate is given by DX. The change in population density is given by

$$\frac{dX}{dt} = growth - output = \mu X - DX \tag{2.B1}$$

At steady state, $\mu = D$; this equality shows how the input rate of substrate controls population growth rate. In principle, chemostats will run until steady state is reached, and this will occur as long as the dilution rate does not become less than the critical dilution rate, D_c, which is the maximum dilution rate at which output balances growth (Equation 2.B2). This becomes an issue when substrate input concentration, S_R, becomes low relative to the specific growth rate of the organism (Harder *et al.*, 1977).

$$D_c = \mu_{max}\left(\frac{S_R}{K_S + S_R}\right) \tag{2.B2}$$

If $S_R \gg K_S$, then $D_c \approx \mu_{max}$, and D_c decreases as S_R decreases relative to K_S.

The substrate concentration in a chemostat depends on the balance between the input rate, the output rate, and how much is converted into biomass:

$$\frac{dS}{dt} = S_R D - SD - \frac{\mu X}{Y} \tag{2.B3}$$

where, in addition to the terms already described, S is the substrate concentration in the chemostat and Y is the growth yield (see Equation 2.10). Substituting the Monod equation (Equation 2.6) into Equation 2.B3 yields, at steady state ($dS/dt = 0$), an expression for S:

$$S = \frac{DK_S}{\mu_{max} - D} \tag{2.B4}$$

The steady state concentration of biomass is given as

$$X = Y(S_R - S) \tag{2.B5}$$

which yields the following expression after substituting from Equation 2.B4:

$$X = Y\left(S_R - K_S\frac{D}{u_{max} - D}\right) \tag{2.B6}$$

Thus, the biomass production and substrate concentrations in a chemostat can be predicted from a basic knowledge of grow parameters (μ_{max} and K_S), dilution rate (D), and input substrate concentration (S_R).

3.3. Growth in nature

Microorganisms are typically isolated from nature in nutrient-rich media and are studied under their optimal growth conditions. Under these conditions, there is a concern that microbial "weeds" are often isolated and that these organisms do not necessarily represent the major populations of microbes in nature. Even if environmentally relevant microbial strains have been isolated, aspects of their physiology and growth characteristics in nutrient-rich laboratory cultures probably differ from their situation in nature. This is because nutrients are much more limiting in the environment, and organisms may adapt physiologically to cope (e.g., Morita, 1997). We can further understand the ecology of prokaryotes in nature if we understand how fast they grow, what factors control their growth and how they adapt physiologically to nutrient stress.

Various methods have been employed to measure microbial growth rates in nature. The most widely used are the incorporation rates of radiolabeled nucleotides or amino acids into the production of DNA, RNA or protein. For example, ^{3}H-labeled thymidine incorporates into DNA, and the rate of incorporation should provide a measure of DNA production rate, which is linked to cell growth (e.g., Findlay *et al.*, 1984; Bell and Riemann, 1989).* Tritium-labeled adenine is incorporated into both DNA and RNA, providing a measure of total nucleic acid production, which is also linked to cell growth (e.g., Karl and Winn, 1984; Karl, 1993). Tritium-labeled leucine is incorporated into proteins, and since proteins make up a relatively constant fraction of bacterial biomass (about 60%), cell growth should be related to leucine uptake rates (e.g., Kirchman, 1992, 1993). All of these methods require assumptions about the ability of microbial populations to incorporate the labeled compound, as well as the relative rates of labeled compound incorporation versus incorporation rates of compound formed within the cell. Careful documentation of compound-specific activity is required, and balanced growth (all cellular constituents are produced at constant relative rates) is usually also assumed. The reader should consult the original literature for more detailed discussion of methodology.

Studies of microbial growth rates for water-column bacteria yield doubling times ranging from days to several months (Moriarty, 1986; Ducklow and Carlson, 1992). In surface marine sediments, doubling times of one to several days have been measured (Karl and Novitsky, 1988), and much longer doubling times are likely deeper in sediments where available substrates become quite limiting. Indeed, by a depth of 10 meters in deep-sea sediments, thymidine incorporation rates of around 100 fmol cm^{-3} d^{-1}

*It has been noted, however, that some important groups of anaerobic prokaryotes do not actively incorporate ^{3}H-labeled thymidine during growth (Wellsbury *et al.*, 1994).

(Parkes *et al.*, 2000) indicate cell doubling times of 1000 days, with a population density of about 10^8 cells cm^{-3} and a nominal thymidine incorporation ratio of 10^9 cells $nmol^{-1}$ thymidine incorporated (Findlay, 1993). At depths greater than 100 meters at the Blake Ridge, and at other deep-sea sites, thymidine incorporation rates of approximately 0.2 fmol cm^{-3} d^{-1} (Parkes *et al.*, 2000; Wellsbury *et al.*, 2000) indicate cell doubling times of over 100 years, with ambient population sizes of around 10^7 cells cm^{-3}, and the same incorporation ratio applied above.

Obviously, microbes in nature are impacted by moderate to severe nutrient limitation. However, substrate limitation occurs as demand outstrips supply, and demand is driven by population size as well as activity. Therefore, nutrient limitation also arises because of overpopulation, when microbes maintain relatively high populations in the face of restricted substrate supply and adapt physiologically to cope with this circumstance. Specific physiological adaptations to low substrate supply are numerous. Commonly, microbes reduce cell size in the face of starvation to increase their surface area to volume ratio, better allowing the efficient fueling of their active cytoplasm (e.g., Novitsky and Morita, 1976). The ribosome and RNA contents of cells also decrease, consistent with a slowing of protein synthesis and overall metabolic function (e.g., Kemp *et al.*, 1993; Morita, 1997). If extreme nutrient limitation is transient, cells may metabolize internal carbon reserves. In some instances, nutrient-limited cells may induce chemotaxis and flagellation (Beveridge, 1989) to help locate scarce substrates if they are unevenly distributed, and high-affinity uptake systems might also be induced to cope with low nutrient supplies (e.g., Jannasch, 1979). Frequently, nutrient-limited cells also become more robust and resistant to environmental extremes such as high pressure, large temperature variations, UV light, and oxidative agents (Morita, 1997). It has also been noted that viable cell numbers in a severely nutrient-limited population might decrease, with the remaining cells still metabolically active but unable to grow (Postgate and Hunter, 1962).

Observations on microbial adaptation to nutrient limitation are conducted on laboratory timescales, which cannot reproduce the slow growth conditions and extreme substrate limitation as is found, for example, in deeply buried sediments. Therefore, the metabolic response of these organisms to nutrient limitation is somewhat uncertain. However, observations demonstrate that approximately 1 to 2% of the total cell numbers as identified through DNA stains such as DAPI (4′,6-diamidino-2-phenylindole) or acridine orange are dividing in marine sediments buried in depths of over 100 m (Parkes *et al.*, 1990, 1994). Furthermore, in these same sediments, ^{3}H-thymidine incorporation can be measured, and radiotracer studies demonstrate biologically mediated sulfate reduction and methanogenesis (e.g., Parkes *et al.*, 2000). With these observations, especially the relatively high

proportion of dividing cells, it seems likely that a high percentage of the total cells are viable and active. Nevertheless, attempts to enumerate viable cells with standard most probable number (MPN) techniques frequently show viable cell numbers several orders of magnitude lower than total cell numbers (e.g., Parkes *et al.*, 1994). This discrepancy likely demonstrates either our inability to find the proper growth media to induce a high proportion of cell growth, or our inability to wait long enough for slow-growing cells to respond physiologically to sudden nutrient-rich conditions.

3.4. Cell numbers and substrate levels in nature

We have seen above that microbes in nature often live under circumstances of nutrient limitation. This limitation certainly arises in part from limited substrate availability, but it also arises from high population numbers sharing the limited resource. In lake and marine water columns cell numbers typically vary between 10^4 and 10^6 cells cm^{-3}, and in surface sediments cell numbers are usually in the range of 10^8 to 10^{10} cells cm^{-3}. Cell numbers decrease with depth in sediments, but not strongly. Even at depths of hundreds of meters in deep-sea sediments, representing millions of years of deposition, cell numbers may still be in the range of 10^6 to 10^7 cells cm^{-3} (Parkes *et al.*, 2000). Microbial population size must represent the balance between growth and death, where growth is controlled by the physiological response of organisms to the available substrate concentrations, and death is controlled by processes such as starvation, viral infection, and grazing.

In what follows, we develop a simple model to explore the major processes controlling microbial population sizes in nature, focusing on acetate as the electron donor. A more complicated carbon flow would necessitate a more complex model, which would probably not alter the main conclusions offered here. We begin by assuming that microbial growth can be expressed by Michaelis-Menten-like kinetics (Equation 2.11):

$$R_G = YV_{max}\frac{SN}{(K_m + S)} \qquad (2.11)$$

where population growth rate (R_G; cells cm^{-3} d^{-1}) is linked to the maximum specific metabolic rate (V_{max}; nmol C $cell^{-1}$ d^{-1}) through the growth yield (Y; cells nmol C^{-1}), substrate concentration (S; nmol C cm^{-3}), half saturation constant (K_m; d^{-1}), and the population size (N; cells cm^{-3}). We assume cell death rate (R_D; cells cm^{-3} d^{-1}) is a simple first-order function of population size (N), with k_D (d^{-1}) the coefficient describing the death rate:

$$R_D = k_D N \qquad (2.12)$$

Overall, the change in population size is the difference between growth and death rates (see also Lovley and Klug, 1986):

$$dP/dt = R_G - R_D = YV_{max}\frac{SN}{(K_m + S)} - k_D N \quad (2.13)$$

Thus far, we have only one equation (Equation 2.13) but two independent variables, population size, N, and substrate concentration, S. We therefore seek a further expression for substrate concentration. The concentration of substrate available to the microbial population will reflect the balance between substrate production rate and substrate consumption rate by the microbes:

$$dS/dt = k_C C - V_{max}\frac{SN}{(K_m + S)} \quad (2.14)$$

The first term expresses the rate of substrate availability, which is first-order with respect to the concentration of organic matter in the environment, C (nmol cm^{-3}), with k_C (d^{-1}) as the carbon oxidation coefficient. This is a normal representation of organic carbon oxidation rate (Berner, 1980). The second term expresses the rate of substrate oxidation by the microbial population, assuming that oxidation rate follows Michaelis-Menten kinetics.

If we assume steady-state ($dN/dt = 0$, and $dS/dt = 0$), Equation 2.13 simplifies to an expression for substrate concentration (Equation 2.15), which, surprisingly, does not depend directly on population size or organic matter reactivity (see also Lovley *et al.*, 1982):

$$S = \frac{k_D K_m}{YV_{max} - k_D} \quad (2.15)$$

To solve for population density, Equation 2.14 is rearranged to yield

$$V_{max}\frac{SN}{(K_m + S)} = k_c C \quad (2.16)$$

This is then substituted into Equation 2.13 to yield

$$N = \frac{YCK_C}{k_D} \quad (2.17)$$

Therefore, the size of a microbial population depends only on the growth yield, Y, the availability of organic carbon, C, carbon reactivity, k_C, and the coefficient describing death rate, k_D.

In what follows, we use Equation 2.17 to rationalize population sizes and substrate levels in natural settings. We assume a spherical cell diameter of

1 μm, a cell density of 1 g cm^{-3}, a cell dry weight of 30% and that 40% of the dry weight is organic carbon. These values yield a cell dry weight of 1.56×10^{-13} g cell^{-1} and a carbon content of 5.2×10^{-6} nmol C cell^{-1}. We further assume that all organic carbon mineralization proceeds through acetate, and with acetate as a carbon substrate, V_{max} values for sulfate reducers range from 830 to 9600 μmol g^{-1} h^{-1}, or, with the cell carbon content above, 3.1×10^{-6} to 36×10^{-6} nmol C cell d^{-1} (Widdel, 1988). Typical growth yields, Y, for acetate-utilizing sulfate reducers range from 4 to 10 g cell dry mass per mol acetate dissimilated (Widdel, 1988), or 0.13 to 0.33 moles of cell carbon per mole acetate. Utilizing the cell carbon content above, 25,000 to 63,500 cells are formed per nmol of acetate used.

We furthermore assume an active organic carbon content in the sediment of 0.5 wt %, which, with a porosity of 0.8 and a dry sediment density of 2.5, yields 2×10^5 nmol C cm^{-3}. Finally, typical K_m values for acetate-utilizing pure cultures of sulfate reducers lie in the range of 64 to 240 μM, while for mixed populations in anoxic sediments, K_m values for acetate utilization are much lower, around 3 to 5 μM (Lovley and Klug, 1986; Widdel, 1988). We will take these mixed population results as most representative, and with a sediment porosity of 0.8, they translate into K_m values of around 2.5 to 4 nmol cm^{-3}. We can now begin to use the equations. We set V_{max}, K_m, Y and C to the values shown in Table 2.2 (see also above). In our first example, we look at marine surface sediments of the continental margin, where values of k_C range from 3×10^{-3} to 3×10^{-4} d^{-1} (Boudreau, 1997), while values of k_D, though largely unexplored, are probably in the range of 0.1 to 0.01 d^{-1}. Taken together, acetate concentrations of 0.06 to 0.6 nmol cm^{-3} are indicated, with population densities of 2×10^8 to 2×10^9 cells cm^{-3} (Table 2.2).

Table 2.2 Values used for modeling substrate concentrations and population densities

$V_{\max}$	2.5×10^{-5} nmol C cell^{-1} d^{-1}
K_{m}	4 nmol$_{\mathrm{acetate}}$ cm^{-3}
Y	30,000 cells nmol$^{-1}_{\mathrm{acetate}}$
k_D	Variable
k_C	Variable
C	Variable

Some results for continental margin scenario:

k_D (yr^{-1})	k_C (yr^{-1})	S (μM)	N (cells cm^{-3})
0.1	0.003	0.06	1.8×10^8
0.01	0.003	0.054	1.8×10^9
0.01	0.0003	0.054	1.8×10^8

Both of these predictions are within the range of observations (see also Chapter 3 for a discussion of substrate concentrations).

With increasing sediment depth, one might anticipate a decrease in the concentration of both organic carbon, C, and growth yield, Y, each of which could lower population numbers. Furthermore, population numbers would also decrease if the death rate constant, k_D, decreased more slowly than the reactivity of organic carbon, k_C. We take deep sediments from the Japan Sea as an example (Parkes *et al*., 2000). As mentioned previously, thymidine incorporation rates deep in these sediments suggest cell-doubling times of about 100 years. If the population is at steady state, then a death rate constant of about 2×10^{-5} d^{-1} is calculated. Sulfate reduction rates measured with radiotracer are very low, approximately 0.002 nmol cm^{-3} d^{-1} (Parkes *et al*., 2000), which translates into a k_C value of about 1×10^{-7} d^{-1}, assuming a reactive C content of about 20,000 nmol cm^{-3} (around 0.05 wt % with a porosity of 0.6). If we retain the value of Y from Table 2.2, then from Equation 2.17 a population density of 2×10^6 cells cm^{-3} is calculated, which is very close to the measured cell numbers (Parkes *et al*., 2000). We also calculate an unreasonably low acetate concentration of around 1.4×10^{-4} nmol cm^{-3}. In deeply buried sediments, and in other natural environments with severe substrate limitation, substrates other than organic carbon may limit growth and activity, and the growth parameters for organisms may differ from our assumed values. Furthermore, in deeply buried deep-sea sediments thermogenic processes increase acetate concentration (Wellsbury *et al*., 2000). All of these factors could elevate acetate concentrations over our predicted values.

4. ENVIRONMENTAL EXTREMES

Microorganisms in nature are found in an amazing spectrum of environments representing extreme departures from average Earth-surface conditions. However, as organisms are usually well adapted to their environment, what seems extreme to us may be quite comfortable for the organisms living there. Therefore, what is extreme is a matter of perspective. In the following, we focus on the most common departures from average Earth-surface conditions and consider some of the special biochemical and physiological adaptations microorganisms use to adapt to these environments. We do not consider all of the various extreme circumstances under which microorganisms can live. The discussion that follows is rather general, focusing on adaptation strategies; examples of specific microorganisms living under extreme conditions can be found in the individual Chapters on elemental cycling.

4.1. Temperature

The growth rates and metabolic rates of microorganisms respond to temperature in a profound way. The response of an individual organism to temperature changes is usually defined with three cardinal temperatures: a minimum growth temperature, an optimal growth temperature, and a maximum growth temperature (Figure 2.5). Typically, the optimal growth temperature is relatively close to the maximum growth temperature, and the steep drop between the two represents the influence of high temperatures on protein and membrane stability. Ultimately, as the optimal temperature is exceeded, repair mechanisms cannot keep up with the damage imposed by the high temperatures. At low temperatures, enzyme systems are slow, and membrane stiffening reduces the activity of membrane-bound transporter enzymes. Growth rates and metabolic rates follow similar temperature responses, although microorganisms can often metabolize at temperatures somewhat outside of their growth temperature range (Figure 2.5) (Isaksen and Jørgensen, 1996).

Increasing growth rates and metabolic rates, as observed in moving from the minimum to the optimal growth temperature, result from increasing

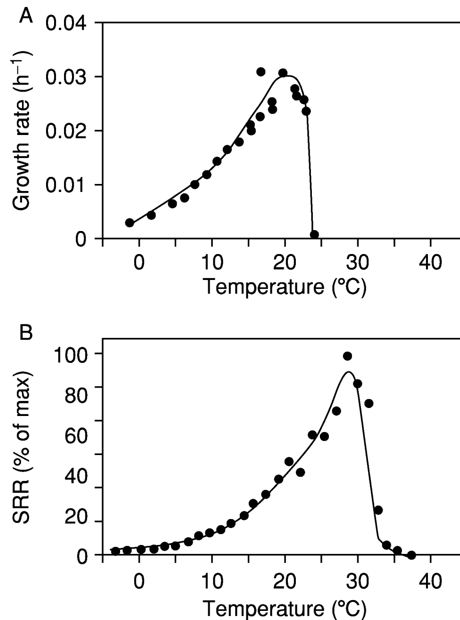

Figure 2.5 Growth rates (A) and sulfate reduction rates (B) as a function of temperature for a psychrophilic sulfate reducer isolated from Norsminde Fjord, Denmark. Redrawn from Isaksen and Jørgensen (1996).

enzyme activity. Within this temperature range both growth rates and metabolic rates are often modeled according to the Arrhenius equation:

$$v = Ae^{-\frac{E_a}{RT}} \tag{2.18}$$

where v is rate (growth rate or metabolic rate), A is a constant, E_a is an apparent activation energy (kJ mol^{-1}), R is the gas constant (8.31 J K^{-1} mol^{-1}), and T is temperature (K). Taking the natural logarithm of Equation 2.18, we find that:

$$\ln v = \ln A - \frac{E_a}{RT} \tag{2.19}$$

Therefore, a plot of ln v vs. $1/T$ will yield a slope of E_a/R, from which E_a can be calculated (Figure 2.6). Note that the Arrhenius equation was originally formulated to represent the kinetic response of pure chemical reactions to temperature, and in this case, E_a has a chemical meaning. When used in microbial systems, calculated E_a values are empirical parameters representing the total metabolic response of an organism to temperature with no grounding in chemical first principles.

A frequently quoted parameter is the Q_{10} response of an organism, which represents the proportional increase in metabolic rate or growth rate with a 10 °C increase in temperature. The Q_{10} response is related to the activation energy (E_a) by the following expression, where T_1 is the reference temperature (°K) and $T_2 = T_1 + 10$:

$$Q_{10} = e^{E_a(T_2 - T_1)/RT_1T_2} \tag{2.20}$$

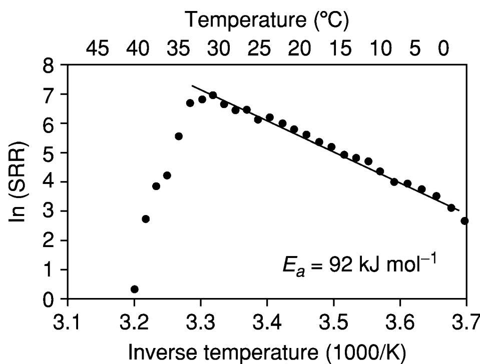

Figure 2.6 1/T vs. ln(sulfate reduction rate) for the same sulfate-reducing culture as in Figure 2.5. From the slope of this relationship, the activation energy, E_a, can be calculated (see Equation 2.18). Redrawn from Isaksen and Jørgensen (1996).

Activation energies for microbial populations, expressing either growth or metabolic rate, generally fall in the range of 50 to 110 kJ mol^{-1}, corresponding to Q_{10} values ranging from around 2 to 5. Note that with a constant E_a, Q_{10} values change slightly through the temperature range of growth.

Taken together, microbes in nature are known to function from sub-freezing temperatures to 121 °C (see also Chapter 1). Organisms with maximum growth rates at <15 °C are known as psychrophiles, organisms with maximum growth rates from 15 °C to 45 °C are known as mesophiles, and organisms with maximum growth rates from 45 °C to 80 °C are thermophiles. Organisms with growth optima above 80 °C are known as hyperthermophiles (Table 2.3). Organisms living at the high and low temperature extremes require special biochemical adaptations in order to survive. For example, psychrophiles must maintain cell membrane fluidity in the face of low temperatures; they do this by modifying the membrane lipid composition

Table 2.3 Nomenclature describing different environmental adaptations

Environmental circumstance	Different adaptations	Notes
Temperature		
	Psychrophile	Max growth <15 °C
	Mesophile	Max growth 15 to 45 °C
	Thermophile	Max growth 45 to 80 °C
	Hyperthermophile	Max growth >80 °C
pH		
	Acidophile	Max growth pH < 5
	Neutrophile	Max growth pH 6 to 8
	Moderate alkaliphile	Max growth pH 8 to 9.5
	Obligate alkaliphile	Max growth pH >9.5
Salt		
	Mild halophile	Max growth 1 to 6% NaCl
	Moderate halophile	Max growth 6 to 15% NaCl
	Extreme halophile	Max growth >15% NaCl
Oxygen		
	Aerobe	
	Obligate	O_2 required
	Facultative	O_2 not required but preferred
	Anaerobe	
	Obligate	Cannot grow with O_2 present
	Facultative	Can tolerate O_2, but grows best without

(e.g., Scherer and Neuhaus, 2002). Specific adaptations include, but are not restricted to, synthesis of a higher proportion of unsaturated fatty acids in the membrane lipids and carbon-chain shortening. Enzymes tend to be more polar, with fewer hydrogen bonds and fewer ion pairs, which reduce hydrophobic interactions between enzyme subunits. Together, these adaptations allow greater enzyme flexibility in the cold. In addition, ribosome structure in psychrophiles is modified compared to mesophiles to aid protein synthesis at low temperatures (Scherer and Neuhaus, 2002).

At the other end of the temperature spectrum, thermophiles, and especially hyperthermophiles, have special adaptations to high temperatures. Compositional and structural changes in enzymes lead to higher thermal stability (e.g., Jaenicke and Sterner, 2002). Proteins have increased numbers of ion pairs and more hydrophobic interiors, and these adaptations, either singly or in combination, help resist unfolding. Nucleic acids tend to denature at high temperatures. For RNA, organisms may respond by increasing the G + C content, which imparts more stability. For DNA, special proteins may be produced that help to stabilize the DNA structure. Fast-acting repair systems are also utilized. In addition, the lipid composition of the cytoplasmic membrane may be heat stabilized by incorporating a high proportion of saturated fatty acids. This is by contrast with the psychrophiles, whose membrane lipids, as discussed above, incorporate short-chained unsaturated fatty acids and chain shortening to increase membrane fluidity.

4.2. pH

Individual organisms generally have a pH tolerance of 2–3 units, and most prokaryotes live with growth optima in the pH range of around 6 to 8. These are known as neutrophiles (Table 2.3). However, acidic environments, including sulfidic hot spring and acidic mine waters, may also house active microbial populations with growth optima at pHs of <6; organisms living under such conditions are known as acidophiles. Indeed, growth is known among iron-oxidizing and heterotrophic *Archaea* at pHs down to zero (e.g., Schleper *et al*., 1995; Edwards *et al*., 2000), which would seem to be the record low pH for microbial growth. Obligate alkaliphiles, living for example in soda lakes and alkaline soils, have optimal growth at pH values above around 9.5 (Krulwich, 2000).

Organisms growing under extremes of pH, both high and low, maintain cytoplasmic pH values within the neutrophilic range, and they face special problems in doing this in the face of strong pH gradients across the cell membrane. Several factors contribute to cytoplasmic pH regulation. The pH-buffering capacity of the cytoplasm itself helps to regulate pH, with buffering coming from the phosphate groups associated with RNA and

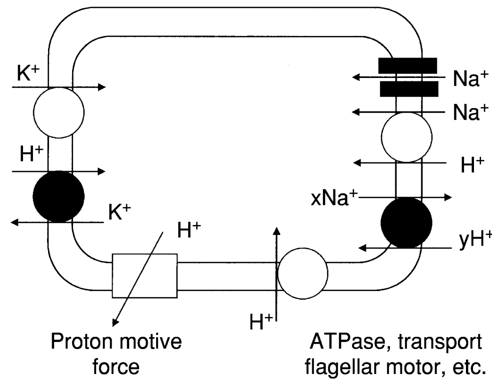

Figure 2.7 The main factors regulating the pH of a cell. The pH-sensitive processes are given by the darkened circles. Protons are translocated across the cell membrane generating a proton motive force used, for example, to generate ATP and to drive flagellar motion. An imbalance in H^+ concentration within the cell cytoplasm can be regulated by the antiport input of H^+ into the cell balanced by K^+ export, where K^+ is regenerated through K^+ import. The K^+/H^+ antiport is pH sensitive, and it is used to regulate cytoplasmic pH in acidophilic organisms. In alkaliphiles pH regulation is controlled by a Na^+ circuit. A pH-sensitive antiport exports Na^+ from the cell, replacing it with H^+. The amount of H^+ imported exceeds the Na^+ exported, to yield acidification of the cell cytoplasm. Na^+ replacement occurs through either a separate symport or a pH-sensitive Na^+ channel. Inspired from Booth (1999).

DNA and protein-associated side-chain amino acids. In addition, for acidophiles, a K^+ ion circuit regulates H^+ entry into the cell, and this circuit is pH sensitive (Figure 2.7). For alkaliphiles the internal pH is lower than the external pH, and these organisms couple the import of H^+ into the cell with the export of Na^+ from the cell (Booth, 1999) (Figure 2.7). For both acidophiles and alkaliphiles, surface-bound proteins are well adapted to the pH of the external environment, and cell membranes are also adapted to the external pH. Thus, when strongly acidophilic prokaryotes are exposed to neutral pH their membranes typically dissolve, demonstrating a strong requirement for low pH in these organisms.

4.3. Salt

All microorganisms maintain lower activities of water (a_w) within their cells compared to the external environment. Thus, water will tend to diffuse into the cell, establishing an osmotic pressure (turgor) that is necessary for cell growth and that must be maintained. Lower cytoplasmic water activity is

established with a higher solute concentration in the cell compared to the external environment, and the solutes used are known as compatible solutes. Compatible solutes are either organic compounds or inorganic ions like K^+, which have several special properties. They must be highly soluble, and they must not interfere with enzyme activity in the cell. When organic compounds are used, they are frequently synthesized within the cell but may also be available from the environment. Obviously, the challenge to produce low water activity within the cell, and to produce high concentrations of compatible solutes, becomes more acute as the salinity of the external environment increases. Despite these hardships, diverse populations of halophilic organisms (see Table 2.3) are found at salinities up to halite (NaCl) saturation, corresponding to about 35% salt by weight.

We consider now how compatible solute choice influences the energetics and microbial ecology of halophilic organisms. When K^+ is used as a compatible solute (members of the aerobic archaeal family *Halocteriaceae*, and *Bacteria* from the order *Halanaerobiales*) (Oren, 1999), only a modest amount of energy is used to accumulate K^+ into the cell, around 1 ATP per 1.5 to 2 moles of KCl. The internal cellular biochemistry must, however, adapt to function in a high salt environment (Oren, 1999). By contrast, when organic compounds are used as compatible solutes, the energetic costs to the organism are huge, as one molecule of compatible solute requires between 30 to 109 ATPs (Oren, 1999). However, with compatible organic solutes, the cell cytoplasm is a relatively low-salt environment, and special biochemical adaptations to high salt are not required. Despite this modest advantage, due to the high-energy cost, organisms utilizing organic compounds as compatible solutes may face problems in obtaining enough energy to grow if they conduct low energy-yielding metabolisms. Microorganisms fitting into this category are methanogens forming methane from acetate or H_2 plus CO_2 (see Chapter 10), nitrifiers, sulfate reducers using acetate, and homoacetogens reducing CO_2 with H_2 to produce acetate (Oren, 1999). Indeed, no organisms conducting these metabolisms have been isolated, nor have their metabolisms been detected, at the high salt concentrations at which more energetic metabolisms are active (Oren, 1999).

5. POPULATION ECOLOGY

We wish to understand the population ecology of microbial ecosystems in the same way we do for macroscopic ecosystems. Thus, we wish to know what populations are present in an ecosystem, the population size, and how populations interact with each other. Basically, we would like to answer the simple question of who is doing what. Unfortunately, we have only a

rudimentary understanding of the species diversity of microbial populations in nature. Part of the problem is that microscopic observations and culturing techniques give only a small glimpse of the diversity of the ecosystem. We know this because DNA extraction and amplification records a far greater diversity, but, unfortunately, we also obtain mostly unknown molecular isolates whose functional role in an ecosystem is unknown. Another part of the problem, as we shall see below, is that even an understanding of diversity does not tell us the activity level of individual population members.

However, the situation is not hopeless. Even though we cannot yet adequately describe the cast of players in a microbial ecosystem or their individual level of activity, we have a reasonably good appreciation for the main processes present, or in other words, the phenotypes represented in the population. Our phenotypic understanding of microbial ecosystems is based on process rate measurements such as carbon fixation rates, sulfate reduction rates, and nitrogen turnover rates, and on chemical profiles that respond to the activity of various metabolic pathways. Furthermore, we can frequently observe the presence of conspicuous population members such as cyanobacteria, sulfide oxidizers, and anoxygenic phototrophs. Our understanding of phenotypic diversity is also based on the exploration of model systems, like industrial fermenters, where many of the main pathways of carbon mineralization have been elucidated (see Chapter 3). Indeed, the phenotypes present in similar microbial ecosystems from around the world are likely quite similar, even though their species composition is unknown and probably quite variable.

In what follows, we summarize important aspects of the population ecology of microbial ecosystems. We discuss microbial diversity, and we describe some of the principal ways in which microbial populations interact. Finally, we explore microbial behavior, and we describe how microbial populations act in coordinated efforts to benefit the individual population, or the entire ecosystem.

5.1. Aspects of microbial diversity

5.1.1. General considerations

We begin with a few comments on microbial diversity. As for populations of macroscopic organisms, microbial populations under stress tend to show less diversity than unstressed populations (Atlas and Bartha, 1998; McCaig *et al.*, 1999). Furthermore, microbial populations living in extreme environments tend to display lower functional diversity, which probably also translates into lower species diversity. For example, photosynthesis is not sustained at temperatures above around 70 °C (Brock, 1994), and as we saw above,

numerous metabolisms such as methanogenesis from CO_2 reduction with H_2 and acetate fermentation, nitrification, and sulfate reduction with acetate appear to be absent at high salt concentrations. Also, recent studies have demonstrated very limited species diversity in low pH acid mine systems (Tyson *et al.*, 2004). Outside of these generalizations, our appreciation for the true diversity of microbial populations in nature is limited by our ability to adequately quantify diversity.

5.1.2. Species diversity from molecular studies

Molecular techniques have provided evidence for far greater microbial diversity than previously imagined (e.g., Ward *et al.*, 1990; Pace, 1997) (see Chapter 1). This is satisfying as we come further in understanding the real diversity of microbial populations in nature, but is also frustrating, as most of the diversity represents unknown organisms. Therefore, we do not know the function of most of these unknown organisms in the ecosystem.

Nevertheless, a number of studies have attempted to evaluate species diversity from a growing body of molecular data, most typically 16S rRNA sequences (e.g., McCaig *et al.*, 1999; Nübel *et al.*, 1999; Hughes *et al.*, 2001). In evaluating diversity, one usually compares 16S rRNA sequences where molecular "species" are defined as different if the sequences are more than 97% different. This is an operational definition of a species, and "species" classified this way are often given the name of operation taxonomic units (OTU). From species abundance and frequency data, diversity indices can be calculated. One such index is the Shannon-Weaver index (H'), which provides an indication of the uncertainty in predicting the identity of a population member if one is chosen at random. The more diverse the population, the more uncertain is the identity of a randomly chosen individual (e.g., Pielou, 1969). The Shannon-Weaver index is expressed as

$$H' = -\sum_{i=1}^{s} p_i \ln p_i \tag{2.21}$$

where s is species and p_i is the proportion of the sample belonging to the ith species. The higher the value for H', the more diverse the sample. Species richness (d) is a further diversity index of interest:

$$d = \frac{s-1}{\log N} \tag{2.22}$$

where s is the number of species and N is the number of individuals. With this index, a higher diversity occurs when there are a large number of species relative to individuals, particularly when N is large.

Of the microbial diversity studies utilizing molecular data, the study of Nübel *et al.* (1999) is noteworthy, as diversity was evaluated for a specific class of organisms, the cyanobacteria. Furthermore, diversity was estimated using not only 16S rRNA sequence data, but also microscopic identification of distinct cyanobacterial morphotypes and the extraction, separation, and identification of individual carotenoids. Eight different microbial mats were explored, and each of the three approaches used to identify cyanobacterial diversity gave a similar picture of diversity when comparing between sites. Other diversity studies have been more general, usually targeting a broad spectrum of the prokaryote world (e.g., McCaig *et al.*, 1999), but nevertheless also showing interesting differences in diversity when comparing between sites.

Even molecular studies, however, do not provide a true picture of microbial diversity, as there are often difficulties in extracting and amplifying DNA fragments from nature. This leads to an underestimate of true diversity. Furthermore, as quantitative amplification procedures are not yet fully developed, minor or even dormant members of the population will appear as

What is a prokaryote species?

According to the concept of species, individuals of a common species can reproduce with each other, but not with individuals of another species. In this way the gene pool of a given species is constantly mixed and shared, and any developing evolutionary innovations are rapidly dispersed. What then about prokaryotes who do not reproduce sexually and whose individuals do not swap genes? It would seem that under these circumstances localized populations could evolve independently of other populations, even those populations originating from the same immediate ancestor, if they are exposed to different environmental circumstances. Indeed, gene acquisition by lateral gene transfer operates locally, which should ensure that individual populations of similar heritage, but spatially displaced, will potentially evolve unique genomes with unpredictable trajectories. In an interesting example, whole genome analysis of two strains of *E. coli* (the pathogenic 0157:H7 strain and the non-pathogen K-12) revealed that about one-third of the genes are different and that many of the gene differences have probably arisen from rather recent gene transfer (Perna *et al.*, 2001).

It would seem that an infinite number of trajectories might be possible from any individual prokaryote, encompassing all possible metabolic capabilities. However, we know from the Tree of Life that a great deal of metabolic relatedness often accompanies phylogenetic relatedness based on SSU rRNA comparisons (see also Chapter 1). We also know that an individual "species" of sulfate reducer (as defined by SSU rRNA sequence), for example, will normally share a remarkably similar physiology (phenotype) to the same "species" from another part of the globe, although the two are countless generations removed. Why is this so? It seems that the answer might be at least partly ecological. We can conjecture that an individual prokaryote "species" is keenly adapted to a particular niche in the environment. If the population representing the species were to acquire too many new traits it might face stiff competition from organisms already better adapted to these particular traits. So, the population, or at least most of it, continues doing what it is best at. In this view, a "species" is stabilized genetically by particulars of environmental adaptation. Nevertheless, as we have seen with the example of *E. coli*, "species" can and do evolve quite different genomes.

important as major population members. Thus, it is difficult to quantify the involvement of individual community members within the ecosystem. Furthermore, most molecular isolates from nature are from organisms not yet cultured and whose physiology is therefore uncertain. This means our understanding of functional diversity lags behind even our understanding of species diversity.

5.1.3. Fluorescent in situ *hybridization (FISH)*

The exploration of community structure with fluorescent *in situ* hybridization (FISH) offers a different type of window into microbial diversity. FISH probes consist of a small stretch of nucleic acid attached to a compound that fluoresces when excited with UV light (Amann *et al.*, 1995). The nucleotide sequence of the probe is chosen to complement a stretch of SSU rRNA to which the FISH probe is hybridized. Under a fluorescence microscope, one sees the ribosomes in individual cells to which the FISH probe has bound. FISH probes are designed from known SSU rRNA sequences, and after examining sequences of related and distant organisms, small stretches that are unique, or nearly unique, to the target organisms of interest can often be identified. Thus, FISH probing provides a straightforward way to differentiate between members of the principal domains in environmental samples. Sequence stretches that are specific to closely related groups of organisms, or even individual species, can also often be identified.

The FISH technique can, in principle, provide a quantitative understanding of population structure, and FISH has yielded some spectacular results. For example, FISH probes have beautifully illustrated the three-dimensional structure of the sulfate-reducing and methanogenic populations believed responsible for anaerobic methane oxidation in marine sediments (Boetius *et al.*, 2000) (see Chapter 10). FISH has also revealed the close physical relationship between ammonia-oxidizing and nitrite-oxidizing chemolithotrophs (Schramm *et al.*, 1996) (see Chapter 7). These two populations are responsible for ammonia oxidation to nitrate. FISH has also revealed the close association between sulfide oxidizers and sulfate reducers as endosymbionts in oligochaete worms (Dubilier *et al.*, 2001).

Despite these successes, and many more, there is still a limit to what FISH probes can provide in defining microbial community structure. For example, many FISH probes are rather unspecific, and they hybridize with organisms outside of the intended group. This, however, is more a problem of probe design than an inherent problem with FISH. Other difficulties with FISH arise from a poor understanding of the diversity of microbes in nature. If probes are used to specify for related groups of organisms they will frequently hybridize with some organisms that have yet to be isolated

(most organisms have not). We cannot be sure whether these uncultured organisms behave similarly to their cultured relatives. Furthermore, since only a small percentage of natural microbial diversity has been identified, we cannot anticipate the appropriate nucleotide sequence, much less the physiology, of organisms that are currently unknown.

5.2. Microbial interactions

From years of careful observation, a wide variety of different types of microbial interactions can be described in nature. The types of interactions are numerous, often complex, and highly interesting. According to the list of interactions presented in Table 2.4, they can be separated into seven general types depending on how the two interacting populations are affected. Furthermore, when the interactions become very intimate, and are obligate for one or both of the populations, another type of interaction, symbiosis, is also observed. We here explore the different types of interactions with examples from the microbial world.

5.2.1. Competition

Competition for available resources, inducing negative effects on the participating populations, is intense in the microbial world. For example, organisms with similar overall physiologies such as sulfate reducers compete with

Table 2.4 Microbial interactions in nature

	Effect of interaction	
Type of interaction	Population 1	Population 2
General		
Competition	−	−
Synergism, syntrophy	+	+
Predation	+	−
Parasitism	+	−
Commensalism	0	+
Amensalism	0	−
Neutralism	0	0
Obligate		
Symbiosis		
Commensalism		
Mutualism		
Parasitism		

After Atlas and Bartha (1998), with modifications.

each other. They can do so by fine-tuning their growth to the particulars of substrate availability and thereby exclude other population members with similar substrate requirements. In chemostat experiments with two populations sharing the same substrate, the population with the highest specific growth rate (a function of both K_s and μ_{max}) eventually will outgrow the other population (Jannasch, 1967). The less successful population will wash out of the chemostat, leaving the successful population behind. The successful population under substrate limitation could be the one with highest substrate affinity (lowest K_s), or when substrate is non-limiting, the population with the highest μ_{max} (Figure 2.8).

As a strategy to increase competitive fitness, prokaryotes in nature tend to be generalists, relying not on one, but on a variety of different substrates (or even types of metabolisms) for survival. In this way, they can compete on several fronts. Population members might also adapt to very specific substrates available only in limited abundance, and for which competition is not keen. In another strategy, members of the population might stay dormant until appropriate conditions materialize. For example, heterotrophs with low substrate affinity, but also high maximum growth rates (μ_{max}), might wait for a sudden input of organic carbon before actively metabolizing. In this way they can, at least temporarily, out-compete organisms with high substrate affinity but lower maximum growth rates (see above).

Organisms with very different physiologies might also compete for substrate. As is shown in Chapter 3, certain anaerobic populations tend to

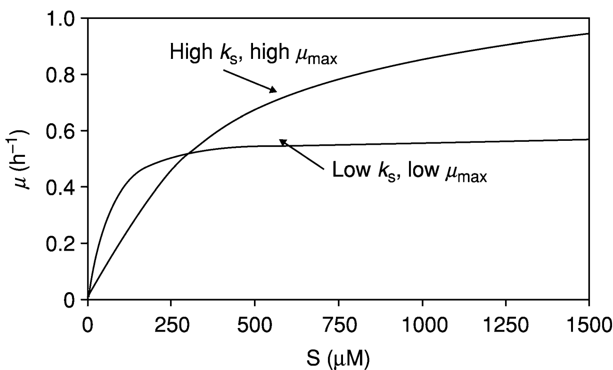

Figure 2.8 Demonstration of how various combinations of maximum growth rate (μ_{max}) and substrate half-saturation constants (K_s) can prove advantageous to growth for different organisms depending on substrate availability. Thus, at low substrate concentrations, the organism with a low K_s (50 μM) and low μ_{max} (0.6 h^{-1}) is favored, while at high substrate concentrations, the organism with a high K_s (400 μM) and high μ_{max} (1.2 h^{-1}) is favored.

survive with the minimal amount of energy available for the maintenance of metabolic functions. Although this may seem like a poorly chosen lifestyle, it serves to remove substrates (such as acetate and H_2) to very low concentration and therefore to exclude other populations with metabolisms gaining less or no energy at these low substrate levels. Thus, iron reducers, for example, can maintain the available substrates in the environment at their survival level, providing insufficient energy for sulfate reducers and methanogens to survive.

Competition might not always be based on energy gain. Some organisms with different metabolisms may compete for a common substrate, with the outcome depending on the physical characteristics of the environment. For example, anoxygenic phototrophic purple sulfur bacteria oxidize sulfide phototrophically (see Chapter 9), and the so-called colorless-sulfur bacteria oxidize sulfide in a chemoautotrophic process by the reaction of sulfide with oxygen or nitrate. Both of these types of organisms are found, for example, closely associated within the sulfide–oxygen interface in microbial mats (Jørgensen and Des Marais, 1986). Whether sulfide oxidation is dominated by phototrophic organisms or non-phototrophic sulfide-oxidizing organisms depends on the availability of light in the near-IR range at the sulfide–oxygen interface. In the microbial mats studied by Jørgensen and Des Marais (1986), IR light penetrated deeper, favoring phototrophic sulfide oxidation, in the mat with the most loosely packed overlying oxygenic phototrophic population.

In another example, chemoautotrophic anammox bacteria, oxidizing ammonia with nitrate, compete with heterotrophic denitrifiers for nitrate in anoxic settings (see Chapter 7). The criteria controlling this competitive interaction in sediments, with steep opposing gradients of critical chemical constituents such as oxygen, nitrate, and ammonia, are not well understood. However, there is a general tendency for anammox to be relatively more important when oxygen penetration is deeper (Thamdrup and Dalsgaard, 2002). In this case, a thicker anoxic nitrate-containing zone is probable, which could benefit anammox bacteria. This is because anammox bacteria are strict anaerobes, whereas many denitrifying strains can withstand micro-oxic conditions. With active carbon mineralization and shallow oxygen penetration, nitrate does not significantly penetrate into the anoxic zone, restricting the activity of anammox organisms. Recent work has shown that the relationship between anammox and denitrification turns from competition to commensalism (see below) in thick anoxic nitrate-containing water columns, such as what might be found in oceanic oxygen minimum zones. Here, the ammonia liberated from organic nitrogen during denitrification is supplied to anammox bacteria, producing a tight coupling between these two processes (Dalsgaard *et al.*, 2003).

5.2.2. Synergism and syntrophy

A synergistic relationship is one in which two members of a population benefit from each other's existence, but the relationship is not obligatory (Table 2.4). When the relationship is one of nutritional interdependence, it is referred to as syntrophy. A good example of syntrophy in microbial populations is the coupling between organisms producing H_2 and those consuming it. This relationship is known as interspecies H_2 transfer (see Chapters 3 and 5). Hydrogen is produced during the fermentation of organic compounds in anoxic environments. As H_2 is a reaction product, its accumulation in the environment renders the fermentation reactions less thermodynamically favorable, until finally, with high enough H_2 partial pressures, fermentation stops altogether (see Chapter 3). However, H_2 is also an excellent electron donor for a variety of anaerobic respiration processes, including metal oxide reduction (Fe and Mn), sulfate reduction, and methanogenesis. Thus, fermentation produces a substrate beneficial to a variety of respiring anaerobes, and consumption of H_2 by these organisms reduces the H_2 partial pressure, allowing the fermentation to continue.

In another example of probable syntrophy, sheathed *Thioploca* spp. filaments contain sulfate reducers of the genus *Desulfonema* (Fossing *et al.*, 1995). *Thioploca* is a sulfide oxidizer utilizing nitrate as the electron acceptor (see Chapter 9). Therefore, *Thioploca* (probably) supplies organic matter to benefit *Desulfonema,* while *Desulfonema* supplies sulfide to benefit the *Thioploca*.

5.2.3. Predation and parasitism

A predator is an organism that feeds on other organisms, and many types of protozoans are the principal predators of prokaryotes in nature. Protists actively engulf prokaryotes (or other food particles) in a process known as phagocytosis, in which a food particle (e.g., prokaryote) is "consumed" in special feeding organelles located at the cell surface. Prokaryotes are delivered to the protist by filter feeding, direct interception, or passive diffusion (Fenchel, 1987). Filter feeding is accomplished by the active transport of water through a filter of cilia, or rigid tentacles on the surface of the protist, which strain small cells (and other food particles) from the environment. In direct interception, fluid flow within the medium carries particles to the surface of the feeding protist. When feeding by passive diffusion, food particles migrate to the protist, either by Brownian motion or through the prokaryote's own motility. Once ingested, food particles form a vacuole, which fuses to membrane-bound enzyme sacs called lysosomes, accomplishing the digestion of the particle.

The impact of protozoans on prokaryote populations can be enormous. In coastal waters, the abundance of flagellated protozoa is sufficient to filter from 10 to 100% of the water column prokaryote population every day (Fenchel, 1987). Such efficient removal keeps prokaryote populations relatively low, allowing the persistence of rapid growth rates. Protozoans are also important feeders of prokaryotes in sediments consisting of well-sorted sand with minimal clay and silt. Here, the protists have ample room to move, and they feed in the interstitial space. However, in fine-grained silts and clays there is insufficient room for protozoan motility, and as a result they are concentrated in the flocculent sediment surface layer, while generally absent in the deeper sediment layers (Fenchel, 1987).

Some prokaryote populations exhibit what may also be considered predatory behavior. For example, various myxobacteria (members of the δ-subdivision of the proteobacteria) thrive in nature by lysing living cells with a variety of hydrolytic exoenzymes, and taking up the cell constituents released. However, these organisms do not depend on living tissues, and they are therefore generally referred to as scavengers rather than predators (Reichenbach and Dworkin, 1992).

Numerous different prokaryotic parasites are disease-causing agents in plants and animals, and some prokaryotes can parasitize other prokaryotic organisms. For example, the gram-negative bacteria *Bdellovibrio* actively hunts and kills its prey to accomplish its parasitic lifestyle. In the attack phase, it is a flagellated non-reproductive cell that enters the periplasmic space of other gram-negative host cells (Figure 2.9). There it loses its flagellum and grows, feeding off the host, into a reproductive septate filament. After growth ceases, the filament separates, lysing the host and releasing a number of individual attack cells, ready to repeat the cycle (Dworkin, 1992).

Viral infection is another sort of parasitism, and it is a major cause of prokaryote mortality in nature. When a virus infects a prokaryote cell, it is known as bacteriophage. Viruses are basically very small (20 to 300 nm diameter) sacs of double- or single-stranded DNA and/or RNA, bound in a protein membrane. As they have no metabolism and cannot replicate on their own, they are not considered life as it is normally defined. In simple terms, viruses use the host's metabolic machinery, in combination with their own genomes, to replicate. To begin a viral attack, the virus needs an appropriate receptor site on which to attach, and after attachment, the viral genome is injected into the host, leaving the protein coat outside of the cell. With a virulent virus, one that destroys the host cell, the host's metabolic machinery is redirected into replicating the viral genome and into the assembly of the structural proteins forming the body of the mature phage particle. Nucleotides forming the host genome may also be harvested into the assembly of new viral genomic material. After the assembly of the phage

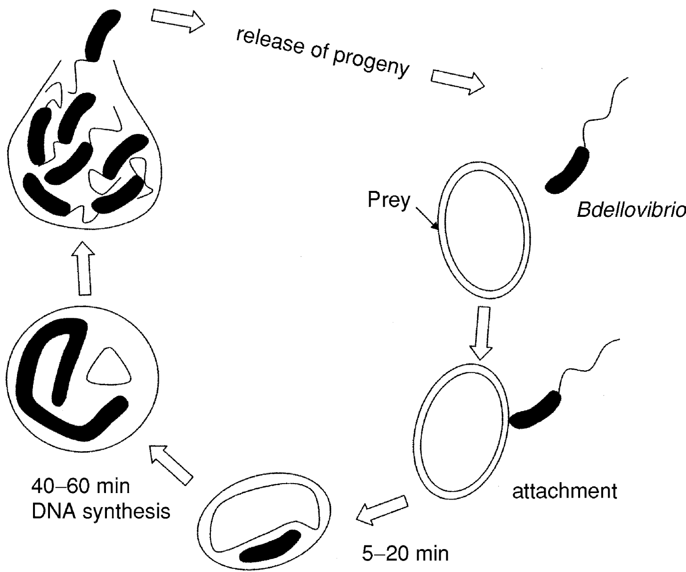

Figure 2.9 Predatory and parasitic lifestyle of *Bdellovibrio bacteriovorus*. *Bdellovibrio* can only reproduce after invasion into a host cell. Here it feeds off the host, grows, and divides into a number of new attack cells, which leave the now decimated host seeking new prey. Inspired from Madigan *et al.* (2003).

particle is complete, the host cell is lysed, releasing the many-formed new phage particles back into the environment.

Temperate viruses form another type of virus–host interaction. After entering into the cell, the virus may integrate into the host chromosome and replicate along with the host chromosome. In this way, the virus is dormant and does not influence the function of the host cell, except perhaps to inhibit the virulent expression of new incoming phage of the same type by expressing special repressor proteins. However, the same virus can enter a virulent stage, at which time the phage genome becomes expressed. New phage particles are formed, resulting in the lysis and death of the host cell. An excellent discussion of viruses and viral infection can be found in Madigan *et al.* (2003).

Active viral infection acts to control microbial population size in nature, and also contributes to the carbon cycle. Thus, after cell lysis following virus attack, cytoplasmic material is released as DOM (dissolved organic matter), which is quickly utilized by other bacteria, stimulating cell growth. This cycle is known as the "viral loop" (Riemann and Middelboe, 2002), and the DOM released as a result of virus infection can constitute a major source of substrate for bacterial growth in planktonic bacterial communities (Middelboe *et al.*, 2002; Riemann and Middelboe, 2002).

5.2.4. Commensalism

Commensalism is the interaction between populations in which one gains from the interaction and the other is unaffected (Table 2.4). In the microbial world, commensalism is mostly related to nutrition, that is, when metabolic products of one microbial population can be used by other microbial populations with no specific gain to the first population. A specific example of commensalism between denitrifiers and anammox bacteria has been presented (see above). Other examples include the production of reduced redox-sensitive species, which, when oxidized, can fuel the growth of other microbial populations. Thus, methanogens produce methane, which can be oxidized by methanotrophs (see Chapter 10), and sulfate reducers produce sulfide, which can be oxidized by a variety of sulfide-oxidizing organisms (see Chapter 9). The microbial loop represents commensalism between eukaryotic algae and aerobic prokaryotic heterotrophs. In this case the algae produce excretion products that are used by the prokaryote population (see Chapter 9).

5.2.5. Amensalism

Amensalism defines a relationship in which the activity of one population is harmful to another. For microbes, this typically results when the products of one type of metabolism are detrimental to another. Examples include the production of oxygen by cyanobacteria, inhibiting anaerobic organisms, or the production of inhibitory organic compounds as metabolic byproducts. For example, ethanol, a fermentation product, is inhibitory to many microorganisms, particularly at higher concentrations. The production of acid during sulfide oxidation, particularly in surface sediments where the pH can be driven very low, creates an extreme environment inhibitory to a wide variety of microorganisms. Some organisms also produce antibiotics that exclude other organisms. We stress, however, that while some microbial populations may be excluded in relationships of amensalism, other populations will thrive. Thus, anaerobes may be excluded in the presence of oxygen, but aerobes will thrive, and while ethanol may be inhibitory to some microbial populations, others can actively use it as a substrate. Also, while low pH might inhibit a great number of different microbial populations, a large number of populations are well adapted to exploit this circumstance.

5.2.6. Neutralism

Neutralism is a lack of interaction between microbial populations. This could occur if populations are spatially separated or if they promote

different types of metabolisms that are not interrelated. For example, in oxic water columns aerobic heterotrophs probably exist without significant interaction with nitrifying bacteria (oxidizing ammonia liberated during organic matter mineralization) or methanotrophs (oxidizing methane from sediment sources, for example). These relationships, however, could become competitive in sediment environments in which oxygen becomes limiting. In general, it is difficult to identify examples of neutralism in nature due to the extensive interrelationships between microbial populations. Thus, at an oxygen–sulfide interface neutralism might be expected, for example, between chemolithoautotrophic organisms such as nitrifiers or sulfide oxidizers and heterotrophs such as sulfate reducers. However, on closer inspection, chemolithoautotrophic organisms produce organic matter that can fuel heterotrophic metabolism. Therefore, even in this situation, a relationship between the populations exists, and strict neutralism probably does not occur.

5.2.7. Symbiosis

Various definitions are used to describe symbiosis, and the borders between syntrophism, parasitism, and commensalism on one hand, and symbiosis on the other hand, are rather blurred. For our purposes, symbiosis is a sustained and intimate physical association between individual species. The interaction need not benefit both partners, as is commonly assumed, but when it does, the association is usually referred to as mutualism. Symbiotic partners can also engage in commensalism, in which one of the partners benefits and the other is unaffected by the relationship. Finally, the symbiosis may be parasitic, with one partner benefiting at the expense of the other. However, cases of parasitic symbiosis are rare in the prokaryote world but are common, for example, among plants. Thus, members of the non-photosynthetic plant family *Orobanchaceae* bury their roots into the tissues of their host plants to obtain nutrition for growth. The parasitic *Orobanchaceae* obviously benefits in this association, while the host is negatively impacted by yielding nutrition to the parasite.

Symbiotic relationships between prokaryote partners are apparently rather rare in nature (Overmann and Schubert, 2002), and while cases of sustained physical associations between prokaryotes can be found (Figure 2.10) (Overmann and Schubert, 2002), the nature of the association is often rather uncertain. A possible example of prokaryote symbiosis is presented above, where sulfate-reducing *Desulfonema* filaments are found within the sheaths of the sulfide oxidizer *Thioploca* sp., although the interdependence and specificity of this relationship have yet to be elucidated. Interdependency also is probable between numerous different consortia involving members of the anoxygenic phototrophic green sulfur bacteria. In this symbiosis, the

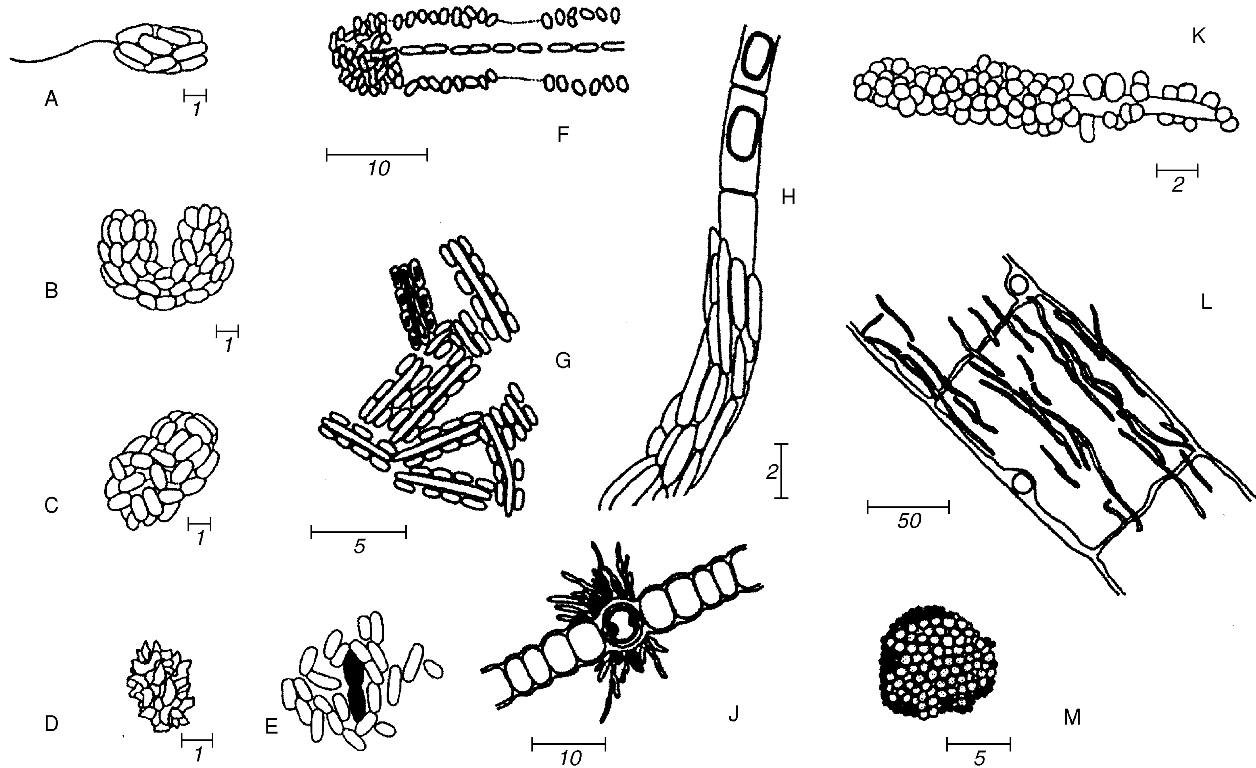

Figure 2.10 Examples of various consortia formed between prokaryotes in nature: (A) morphotype of *Chlorochromatium aggregatium* and *Pelochromatium roseum*; (B) *Chlorochromatium glebulum*; (C) morphology of *Chlorochromatium magnum* and *Pelochromatium roseo-viride*; (D) morphotype of *Chlorochromatium lunatum* and *Pelochromatium selenoides*;

phototroph forms the epibiont, completely covering a central bacterium, which at least in some instances comes from the β-subdivision of the proteobacteria (Overmann and Schubert, 2002) (see also Chapter 9). The exact relationship between these two consortia partners is not clear, so it is not known whether they engage in commensalism or mutualism. However, there is obvious signaling between the two partners, as the consortia exhibit a tactic response to the wavelength optima of the phototrophic partner, but motility is provided only by the flagellum of the central bacterium (see Chapter 9) (Overmann and Schubert, 2002).

Mutualistic symbiotic relationships between prokaryotes and eukaryotes are quite common. Cyanobacteria (as well as algae) join forces with fungi to form lichens. In this association, the phototroph is the primary producer, supplying organic matter and fixed nitrogen (when the phototroph is a cyanobacterium), and the fungus is the consumer, supplying nutrients back to the phototroph for further utilization. The fungus also provides protection for the phototroph. The interactions between the phototroph and fungus are quite specific and distinctive, and the lichens have their own genus and species names. Other mutualistic relationships include the partnerships formed from endosymbiotic sulfide-oxidizing bacteria and various animals including those from hydrothermal vents (see Chapter 4), marine oligochaetes (Dubilier *et al.*, 2001), and bivalves (Gros *et al.*, 2000). These relationships may involve very specific physiological adaptations on the part of the animal to house and nurture the prokaryote hosts. For example, the deep-sea hydrothermal vent tube worm *Riftia pachytila* has evolved without a mouth or intestinal tract, and it obtains its nutrition from sulfide-oxidizing endosymbionts (see Chapter 4), with active transport systems for O_2 and sulfide to fuel the sulfide-oxidizing population. Mutualistic symbiosis is also found between prokaryotes and protozoans, where protozoan populations may house, for example, methanogenic partners as endosymbionts (Fenchel and Findlay, 1995). Hydrogen is produced by fermentation within the hydrogenosomes of the protist, and it is consumed by the methanogen,

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(E) *Chlorochromatium aggregatium* after disaggregation revealing the central bacterium; (F) *Cylindrogloea bacterifera*; (G) *Chloroplana vacuolata*. Gas vacuolation of both the green sulfur bacteria and the central colorless bacteria is shown for only a few of the cells; (H) consortium from the hindgut of the termite *Reticulitermes flavipes*. Upper portion reveals the chain of central bacteria containing endospores; (J) *Anabena* sp. filament with chemotrophic bacteria covering a heterocyst; (K) dental plaque; (L) *Thioploca* sp. filament covered with sulfate-reducers of the genus *Desulfonema*; (M) archaeal-bacterial consortia where the central cluster of Archaea is covered by a layer of sulfate reducers. This consortia is believed to regulate the anaerobic oxidation of methane. Scale bars are in μM. From Overmann and Schubert (2002). Reproduced with permission.

providing an obvious food source for the methanogen. The methanogen removes H_2, thus favoring the fermentation of the hydrogenosome. Consistent with this interdependence, if methanogenesis is inhibited, the growth rate of the protist may suffer, particularly if it is large (Fenchel and Findlay, 1995).

Strombidium purpureum is a protozoan housing a purple non-sulfur phototrophic bacterium (Fenchel and Bernard, 1993). The phototroph utilizes H_2 produced by the protist, obviously benefiting the phototroph, but also the protist by lowering the H_2 partial pressure, favoring continued fermentation. The phototroph may also provide a food source to the protist. In the dark, the protist seeks low oxygen levels of 1 to 4% air saturation, where the phototroph oxidizes H_2 and fatty acids by oxidative phosphorylation (see Chapter 4). Therefore, the phototroph can remain metabolically active day and night, utilizing two different metabolisms. This unusual behavioral adaptation greatly expands the environmental range of the protist (Fenchel and Findlay, 1995).

Some ciliates may also house sulfate reducers as ectosymbionts, attached to external cell surfaces (Fenchel and Findlay, 1995). The sulfate reducers apparently use substrates (e.g., H_2, acetate) coming from the ciliate, and the relationship between the two organisms would appear to be one of commensalism. The sulfate reducers gains from the association with the ciliate, and the cost or gain to the ciliate is less obvious.

5.3. Horizontal gene transfer

The transfer of genetic material between microbes in nature alters the genome of host populations and promotes evolutionary change. As explored in Chapter 1, horizontal gene transfer has been a prominent mode of genome modification through the history of life. Horizontal gene transfer can provide new metabolic possibilities to recipient organisms, but at the same time, it complicates our efforts to reconstruct the history of life from single gene trees such as those constructed from SSU rRNA sequences. Given the potential significance of horizontal gene transfer in building microbial genomes, here we briefly overview some of the pathways by which genetic material may be transferred between prokaryotes in nature.

Some prokaryotes are able to uptake DNA from the environment in a process known as transformation. Cells can only uptake DNA when they are competent, and in some cases competence is induced by a quorum-sensing circuit (see below) when cell numbers become high enough. The DNA incorporated may come from the lysis of other cells after viral attack or starvation. After the DNA is taken up by the competent cell it may be incorporated into the host genome.

Viral infection is another vector for DNA transfer between microorganisms, in a process known as transduction. In this case, the genome of a virus particle incorporates some DNA from a host. The virus particle may incorporate either specific or nonspecific stretches of host DNA, replacing some of the viral DNA. There is the possibility that this DNA can be incorporated into a new host during viral infection, particularly with a temperate virus. There is a low probability of DNA transfer by transduction, as the probability of incorporating part of the host genome into a virus particle is low, and the probability of permanently incorporating some of this prokaryote DNA into another host is also low. Nevertheless, this transfer mechanism can occur, and given the astronomical number of viral infections in prokaryotes in nature, it is probably not uncommon.

Another pathway of gene transfer is a process called conjugation, in which plasmids from one cell are transferred to another, and the genetic material of the plasmid becomes incorporated into the host genome. Plasmids are double stranded, normally circular stretches of DNA existing in the cell cytoplasm independent of the cell chromosome. Plasmids accomplish numerous important functions for the cell, including the production of antibiotics as well as antibiotic resistance, and they can also code for important parts of carbon metabolism. Plasmids are transferred between cells during conjugation by cell-to-cell contact, and the process of conjugation is encoded by the plasmid itself. Conjugation can be a very efficient pathway for genetic material exchange. For example, some plasmids transfer antibiotic resistance between cells during cell-to-cell contact, and others transfer virulence. In principle, any process encoded by a plasmid can be transferred to other members of the same population, or even different distantly related populations (see Madigan *et al.*, 2003, for a full discussion).

6. SOCIAL BEHAVIOR AND CELL DIFFERENTIATION

We can view social behavior as interactions between population members that benefit the population (Crespi, 2001). For example, sulfide oxidizers of the genus *Thiovulum* can attach to solid surfaces with a slime thread (see Chapter 9) and spin rapidly around this tether, enhancing the transport of oxygen and sulfide to the whole *Thiovulum* population. This is a specific behavior enhancing the metabolic activity of the population. Many individual populations can also differentiate both functionally and morphologically in ways to benefit the whole population (e.g., Shapiro, 1998). For example, a number of filamentous cyanobacteria differentiate a portion of their cells into special heterocysts, where nitrogen fixation occurs. With this

physiological (and morphological) adaptation, the cyanobacterial filaments can meet their own fixed nitrogen needs.

Another example of morphological differentiation is found among members of the myxobacteria (see also above), which are aerobic heterotrophs commonly found in soils, living among decaying vegetation and animal dung (e.g., Reichenbach and Dworkin, 1992). In their vegetative state myxobacteria are motile rod-shaped cells with gliding motility. Under nutrient stress, they undergo an impressive and complex process of cell differentiation forming fruiting bodies, which are frequently stalked (Figure 2.11). Complex signaling and cell communication coordinate the formation of fruiting bodies. The process begins as cells aggregate together, forming mounds, after which morphogenesis occurs. This includes the secretion of a slime stock (in species that produce such a stock) and the migration of cells to the top of the stock where the head of the fruiting body is formed. Here, cells differentiate into myxospores, which are sometimes encased in walled structures known

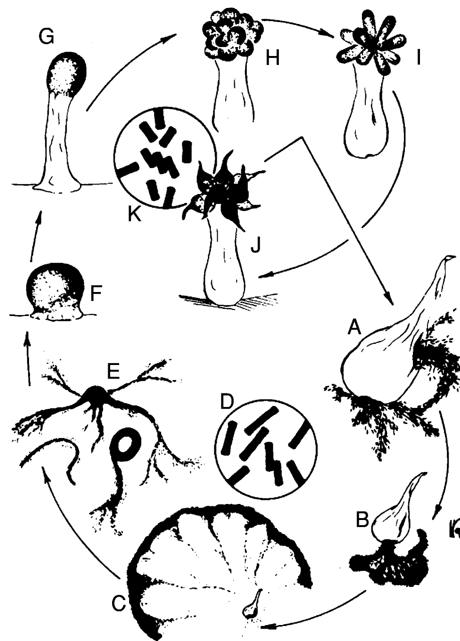

Figure 2.11 Morphogenesis of the myxobacterium *Chondromyces apiculatus*. (A) germinating sporangiole; (B) and (C) swarm colony development; (D) vegetative cells; (E) aggregation of vegetative cells within the swarm; (F) knob formation stage; (G) excretion of slime stalk, with cells concentrating in the terminal knob; (H) terminal mass begins to differentiate; (I) club-like structures form; (J) turnip-shaped sporangioles form; (K) myxospores. From Reichenbach and Dworkin (1992), with permission.

as cysts. Fruiting body cells exhibit enhanced resistance to environmental extremes such as drying and UV radiation. When conditions again become favorable for growth, the sporangiole holding the myxospores ruptures, releasing the myxospores, and the vegetative cycle begins. Yet another example of cell differentiation and community behavior among single populations is colony development by *E. coli*, in which different zones within the colony display cells of unique shape, size, and patterns of arrangement (Shapiro, 1998).

Some individual populations also release and detect chemical signaling molecules, known as autoinducers, in a process called quorum sensing (e.g., Miller and Bassler, 2001; Schauder and Bassler, 2001). As populations grow past a minimum size, autoinducers reach a threshold concentration at which genes are expressed controlling many types of microbial behavior, including luminescence, virulence, antibiotic production, and biofilm formation (Schauder and Bassler, 2001). In general, the quorum-sensing circuit involves the production of a specific protein that in turn produces an autoinducer compound that diffuses freely across the cell membrane. The autoinducer compound accumulates in solution at a concentration proportional to microbial numbers and density. The autoinducer combines with specific receptor proteins within the cell when a critical high concentration of autoinducer is reached. At this point the receptor protein triggers the induction of gene expression (Figure 2.12). In this way, populations can coordinate

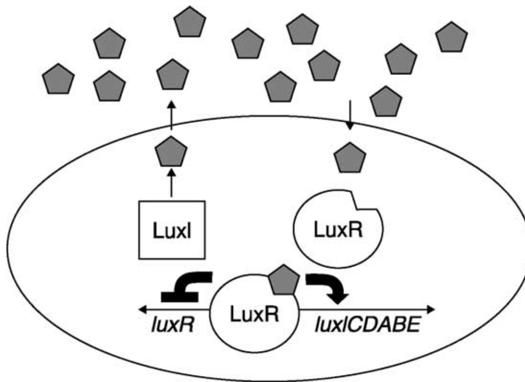

Figure 2.12 Quorum-sensing circuit for *Vibrio fischeri*. Autoinducer (pentagons) accumulates in solution after production by *LuxI*. The concentration of autoinducer is sensed by *LuxR*, which, at a threshold level, activates the *LuxICDABE* operon, which initiates bioluminescence. There is a positive feedback through *LuxICDABE,* causing an increase in autoinducer production (through increasing *LuxI* expression) and increasing, ultimately, bioluminescence. To control light production, the *LuxR*-autoinducer complex also inhibits the expression of *LuxR*, providing a necessary negative feedback. Inspired from Bassler and Miller (2001).

gene expression at cell densities that are advantageous to the population (Miller and Bassler, 2001; Schauder and Bassler, 2001).

The first demonstration of quorum sensing was with the bioluminescent marine bacterium *Vibrio fischeri*, which lives in symbiotic association with various marine animals (Nealson and Hastings, 1979). An example is the association between *V. fischeri* and the nocturnal squid *Euprymna scolopes* (Ruby, 1996; Bassler and Miller, 2001). *V. fischeri* lives within a special light organ on the underside of the squid, and the squid balances the illumination from *V. fischeri* to match the illumination from the moon so that the squid casts no shadow on the sea bottom. This helps protect the squid from predators. The squid gains by having a beneficial source of light, and *V. fischeri* gains from a ready source of nutrients within the light organ of the squid. Quorum sensing controls the bioluminescence from *V. fischeri*. Within the light organ of the squid, the autoinducer compounds produced by *V. fischeri* accumulate to a concentration high enough to induce bioluminescence. By contrast, when *V. fischeri* is present in the environment outside of the squid light organ (the squid sheds the bacteria at sunrise every morning), it produces no bioluminescence because the population density is too small. As a result, autoinducer concentrations are too low to activate bioluminescence gene expression.

In another example of quorum sensing, the human pathogen *Pseudomonas aeruginosa* uses quorum sensing to regulate the expression of a variety of virulence factors that interfere with protein synthesis and promote host tissue destruction (Parsek and Greenberg, 1999; Bassler and Miller, 2001). Presumably, high population densities give the best opportunity for *P. aeruginosa* to infect its host successfully. Numerous other examples of quorum sensing may also be found in nature, and this may indeed be a fundamental aspect of microbial communication (Bassler and Miller, 2001).