ENVIRONMENTAL FACTORS AFFECTING GROWTH

A suitable growth medium must contain all the nutrients required by the organism to be cultivated, and such factors as pH, temperature, and aeration must be carefully controlled. A liquid medium is used; the medium can be gelled for special purposes by adding agar or silica gel. Agar, a polysaccharide extract of a marine alga, is uniquely suitable for microbial cultivation
because it is resistant to microbial action and because it dissolves at 100°C but does not gel until cooled below 45°C; cells can be suspended in the medium at 45°C and the medium quickly cooled to a gel without harming them.

Nutrients

On the previous pages, the function of each type of nutrient is described, and a list of suitable substances presented. In general, the following must be provided: (1) hydrogen donors and acceptors,
about 2 g/L; (2) carbon source, about 1 g/L; (3) nitrogen source, about 1 g/L; (4) minerals: sulfur and phosphorus, about 50 mg/L of each, and trace elements, 0.1–1 mg/L of each; (5) growth factors: amino acids, purines, and pyrimidines, about 50 mg/L of each, and vitamins, 0.1–1 mg/L of each.

Hydrogen Ion Concentration (pH)

Most organisms have a fairly narrow optimal pH range. The optimal pH must be empirically determined for each species. Most organisms **(neutralophiles)** grow best at a pH of 6.0–8.0, although some forms **(acidophiles)** have optima as low as pH 3.0, and others **(alkaliphiles)** have optima as high as pH 10.5.

Microorganisms regulate their internal pH over a wide range of external pH values by pumping protons in or out of their cells. Acidophiles maintain an internal pH of about 6.5 over an external range of 1.0–5.0, neutralophiles maintain an internal pH of about 7.5 over an external range of 5.5–8.5, and alkaliphiles maintain an internal pH of about 9.5 over an external range of 9.0–11.0. Internal pH is regulated by a set

of proton transport systems in the cytoplasmic membrane, including a primary, ATP-driven proton pump and an Na+/H+ exchanger. A K+/H+ exchange system has also been proposed to contribute to internal pH regulation in neutralophiles.

Temperature

Different microbial species vary widely in their optimal temperature ranges for growth (Figure 5-2): **Psychrophilic** forms grow best at low temperatures (–5–15°C) and are usually found in such environments as the Arctic and Antarctic regions; **psychrotrophs** have a temperature optimum between 20°C and 30°C but grow well at lower temperatures. They are an important cause of food spoilage. **Mesophilic** forms grow best at 30–37°C, and most **thermophilic** forms grow best at 50–60°C. Some organisms are **hyperthermophilic** and can grow at well above the temperature of boiling water, which exists under high pressure in the depths of the ocean. Most organisms are mesophilic; 30°C is optimal for many free-living forms, and the body temperature of the host is optimal for symbionts of warm-blooded animals.

The upper end of the temperature range tolerated by any given species correlates well with the general thermal stability of that species' proteins as measured in cell extracts. Microorganisms share with plants and animals the **heat-shock response**, a transient synthesis of a set of "heat-shock proteins," when exposed to a sudden rise in temperature above the growth optimum. These proteins appear to be unusually heat resistant and to stabilize the heat-sensitive proteins of the cell.

The relationship of growth rate to temperature for any given microorganism is seen in a typical Arrhenius plot (Figure 5-3). Arrhenius showed that the logarithm of the velocity of any chemical reaction (log k) is a linear function of the reciprocal of the temperature (1/*T*); because cell growth is the

result of a set of chemical reactions, it might be expected to show
this relationship. Figure 5-3 shows this to be the case over the normal range of temperatures for a given species; log k decreases
linearly with 1/*T*. Above and below the normal range, however, log k drops rapidly, so that maximum and minimum temperature values are defined.
Beyond their effects on growth rate, extremes of temperature kill microorganisms. Extreme heat is used to sterilize preparations
(see Chapter 4); extreme cold also kills microbial cells, although it cannot be used safely for sterilization. Bacteria also exhibit a phenomenon called **cold shock**, which is the killing of cells by rapid—as opposed to slow—cooling. For example, the rapid cooling of *Escherichia coli* from 37°C to 5°C can kill 90% of the cells. A number of compounds protect cells from either freezing or cold shock; glycerol and dimethyl sulfoxide are most commonly used.

Aeration

The role of oxygen as hydrogen acceptor is discussed in Chapter 6. Many organisms are **obligate aerobes**, specifically requiring oxygen as hydrogen acceptor; some are **facultative anaerobes**, able to live aerobically or anaerobically; some are **obligate anaerobes** requiring a substance other than oxygen as hydrogen acceptor and are sensitive to oxygen inhibition; some are **microaerophiles,** which require small amounts of oxygen (2%–10%) for aerobic respiration (higher concentrations are inhibitory); and others are **aerotolerant anaerobes,** which are indifferent to oxygen. They can grow in its presence, but they do not use it as a hydrogen acceptor (Figure 5-4).
The natural by-products of aerobic metabolism are the reactive compounds hydrogen peroxide (H_2O_2) and superoxide (O_2^-). In the presence of iron, these two species can generate hydroxyl radicals (•OH), which can damage any biologic

macromolecule:

$$O_2^- + H_2O_2 \xrightarrow{Fe^{++}/Fe^{3+}} O_2 + OH^- + {}^{\bullet}OH$$

Many aerobes and aerotolerant anaerobes are protected from these products by the presence of superoxide dismutase, an enzyme that catalyzes the reaction

$$2O_2^- + 2H^+ \rightarrow O_2 + H_2O_2$$

and by the presence of catalase, an enzyme that catalyzes the reaction

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

Some fermentative organisms (eg, *Lactobacillus plantarum*) are aerotolerant but do not contain catalase or superoxide dismutase. Oxygen is not reduced, and therefore H2O2 and O2
– are not produced. All strict anaerobes lack both superoxide dismutase and catalase. Some anaerobic organisms (eg, *Peptococcus anaerobius*) have considerable tolerance to oxygen as a result of their ability to produce high levels of an enzyme (NADH oxidase) that reduces oxygen to water according to the reaction

The Medium

The technique used and the type of medium selected depend on the nature of the investigation. In general, three situations may be encountered: (1) One may need to raise a crop of cells of a particular species that is on hand, (2) one may need to determine the numbers and types of organisms present in a given material, or (3) one may wish to isolate a particular type of microorganism from a natural source.

Isolation of Microorganisms in Pure Culture

To study the properties of a given organism, it is necessary to

handle it in pure culture free of all other types of organisms. To do this, a single cell must be isolated from all other cells and cultivated in such a manner that its collective progeny also remain isolated. Several methods are available.

A. Plating

Unlike cells in a liquid medium, cells in or on a gelled medium are immobilized. Therefore, if few enough cells are placed in or on a gelled medium, each cell will grow into an isolated colony. The ideal gelling agent for most microbiologic media is **agar**, an acidic polysaccharide extracted from certain red algae. A 1.5–2% suspension in water dissolves at 100°C, forming a clear solution that gels at 45°C. Thus, a sterile agar solution can be cooled to 50°C, bacteria or other microbial cells added, and then the solution quickly cooled below 45°C to form a gel. (Although most microbial cells are killed at 50°C, the time course of the killing process is sufficiently
slow at this temperature to permit this procedure; see Figure 4-3.) Once gelled, agar will not again liquefy until it is heated above 80°C, so that any temperature suitable for the incubation of a microbial culture can subsequently be used.

In the **pour-plate method**, a suspension of cells is mixed with melted agar at 50°C and poured into a **Petri dish**. When the agar solidifies, the cells are immobilized in the agar and grow into colonies. If the cell suspension is sufficiently dilute, the colonies will be well separated, so that each has a high probability
of being derived from a single cell (Figure 5-5). To make certain of this, however, it is necessary to pick a colony of the desired type, suspend it in water, and replate. Repeating this procedure several times ensures that a pure culture will be obtained.

Alternatively, the original suspension can be streaked on an agar plate with a wire loop (**streak-plate technique**). As the streaking continues, fewer and fewer cells are left on the loop, and finally the loop may deposit single cells on the agar (Figure 5-6). The plate is incubated, and any well-isolated

colony is then removed, resuspended in water, and again streaked on agar. If a suspension (and not just a bit of growth from a colony or slant) is streaked, this method is just as reliable as and much faster than the pour-plate method.

In the **spread plate technique**, a small volume of dilute microbial suspension containing ca 30–300 cells is transferred to the center of an agar plate and spread evenly over the surface with a sterile bent-glass rod. The dispersed cells develop into isolated colonies. Because the number of colonies should equal the number of viable organisms in a sample, spread plates can be used to count the microbial population.

B. Dilution

A much less reliable method is that of extinction dilution. The suspension is serially diluted, and samples of each dilution are plated. If only a few samples of a particular dilution exhibit growth, it is presumed that some of the colonies started from single cells. This method is not used unless plating is for some reason impossible. An undesirable feature of this method is that it can only be used to isolate the predominant type of organism in a mixed population.