8 Pure Cultures and Cultural Characteristics

OUTLINE **Natural Microbial Populations (Mixed Cultures)**

Selective Methods
Chemical Methods of Selection • Physical Methods of Selection • Biological Methods of Selection • Selection in Nature

Pure Cultures
Methods of Isolating Pure Cultures

Maintenance and Preservation of Pure Cultures
Methods of Maintenance and Preservation • Culture Collections

Cultural Characteristics
Colony Characteristics • Characteristics of Broth Cultures

In natural environments a single kind of bacterium, i.e., a bacterial species, usually occurs as only one component of a large and complex population containing many other species. To study the characteristics of one species, that species must be separated from all the other species, i.e., it must be **isolated** in pure culture. However, before attempting isolation, it is often helpful to use a **selective method** first. Such a method can increase the relative proportion of the desired species in the population so that it can be more easily isolated. Once obtained, a pure culture can be maintained or preserved in a **culture collection.** Different species of bacteria growing on the same kind of medium may appear quite different; thus knowledge of the appearance, or the **cultural characteristics,** of a species is useful for the recognition of certain kinds of bacteria and may also serve as an aid to the identification of species. In this chapter we shall describe methods for selection, isolation, and preservation of bacteria, as well as their cultural characteristics on various media.

MICROBIAL IONS (MIXED S)

The microbial population in our environment is large and complex. Many different microbial species normally inhabit various parts of our bodies, such as the oral cavity, the intestinal tract, and the skin. These microbes may be present in extremely large numbers. For example, a single sneeze may disperse from 10,000 to 100,000 bacteria. One gram of feces may contain 10^{11} bacteria. Our environment—air, soil, water—likewise consists of mixed populations of

bacteria plus other microbes. In fertile garden soil, microorganisms may number several billions per gram and include many species of bacteria, fungi, algae, and protozoa. A study of the microorganisms in these habitats requires knowledge of the specific microbes present. This, in turn, requires unraveling the complex mixed population into pure cultures of separate, distinct species.

LECTIVE METHODS

A particular bacterial species is often present in small numbers compared to the total population of a mixed culture. Moreover, the species may be one that grows less rapidly on ordinary culture media than other species. In order to achieve its isolation into pure culture, it is helpful—and often necessary—to first achieve an increase in the relative number of the species, preferably to the point where the species becomes the numerically dominant component of the population. This can be accomplished by the use of selective methods. These methods favor the growth of the desired species while discouraging, or even killing, the other organisms present in the mixed culture. Chemical, physical, or biological methods are used in order to achieve selection of a particular kind of bacterium.

Chemical Methods of Selection

Use of a Special Carbon or Nitrogen Source

One type of chemical method is to provide in the culture medium a substrate, i.e., a single carbon or nitrogen source, that can be used only by the species being sought (Fig. 8-1). This particular kind of selection is often referred to by a special name, enrichment. For example, if we wish to isolate, from soil, bacteria capable of utilizing a very complex organic compound like α-conidendrin, a constituent of wood, we find that when we inoculate a medium such as nutrient agar directly with the soil sample, our chances of finding α-conidendrin–utilizing bacteria will be very limited. There are so many other rapidly growing bacteria present that the more slowly growing kind we wish to obtain will be soon overgrown. Consequently, we prepare a liquid enrichment medium in which α-conidendrin is the sole source of carbon. Under these conditions, only organisms capable of utilizing this compound will be able to grow well. However, it is important to recognize that other bacteria may be able to grow to some extent by utilizing organic compounds made by the conidendrin-utilizing

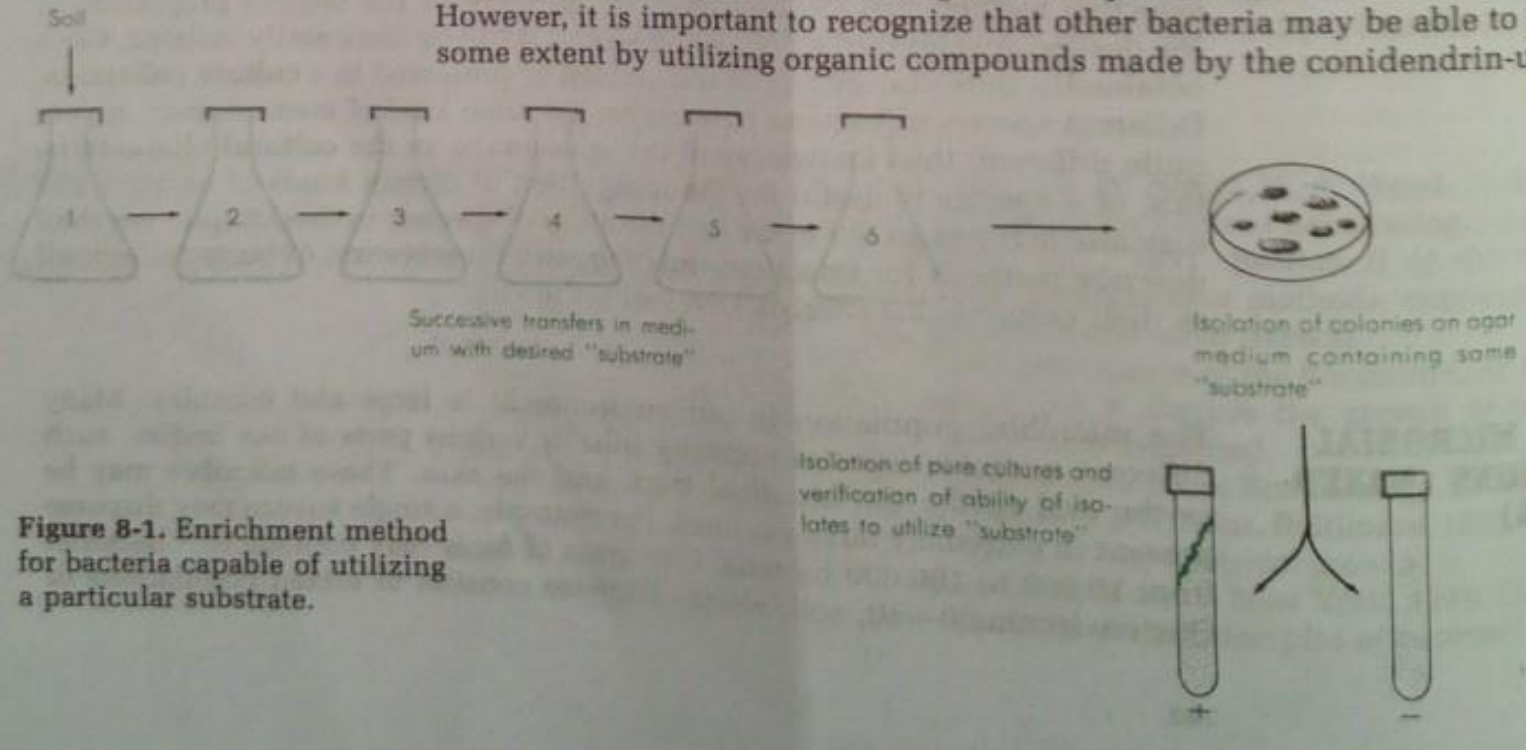

Figure 8-1. Enrichment method for bacteria capable of utilizing a particular substrate.

organisms and that the method is not completely specific. As another example, if we wish to select for nitrogen-fixing bacteria, nitrogen gas (N_2) can be supplied as the sole nitrogen source, since only nitrogen-fixing bacteria will be able to grow well. Other bacteria may grow, but to a lesser degree, by using the nitrogenous products made by the nitrogen-fixers.

se of Dilute Media

Certain aquatic bacteria, such as *Caulobacter* species, are capable of growing with very low levels of carbon or nitrogen sources. Consequently, one way to select for such bacteria is to inoculate a mixed culture into a very dilute medium, e.g., a broth containing only 0.01 percent peptone. The medium must have low enough levels of nutrients that other kinds of organisms will not be able to grow well in it.

Jse of Inhibitory or Toxic Chemicals

The addition of low levels of certain chemicals, such as dyes, bile salts, salts of heavy metals, or antibiotics, to culture media can be useful for the selection of certain kinds of bacteria. The following are examples of this type of selection:

1 Many Gram-negative bacteria can grow in the presence of low concentrations of various dyes that inhibit the growth of Gram-positive bacteria. Similarly, intestinal bacteria can grow in the presence of bile salts such as sodium deoxycholate, whereas nonintestinal bacteria are usually inhibited. Consequently, a medium containing crystal violet dye plus sodium deoxycholate will allow Gram-negative intestinal bacteria to grow but will inhibit most other kinds of bacteria. An example of such a medium is MacConkey agar, which is widely used to select for Gram-negative intestinal pathogens such as *Salmonella* and *Shigella* species.
2 *Campylobacter jejuni* is a frequent cause of diarrhea in humans, yet diarrheic stool samples contain many other kinds of bacteria that interfere with the isolation of this species. By incorporating certain antibiotics or other chemotherapeutic agents, such as vancomycin, polymyxin, and trimethoprim, into the culture medium, most of these contaminants can be inhibited without affecting the growth of *C. jejuni*.

Physical Methods of Selection

To select for endospore-forming bacteria, a mixed culture can be heated to 80°C for 10 min before being used to inoculate culture media. Vegetative cells will be killed by this treatment but endospores will survive and subsequently germinate and grow.

Heat Treatment

Incubation Temperature

To select for psychrophilic or psychrotrophic bacteria, cultures are incubated at low temperatures, e.g., 0 to 5°C. For selection of thermophiles, a high incubation temperature is used, e.g., 55°C.

pH of the Medium

To select for acid-tolerant bacteria, a low-pH medium can be used. For example, to select for the lactobacilli present in cheddar cheese, the pH of the medium is maintained at 5.35 with an acetic acid/acetate buffer; other organisms in the cheese cannot grow well at such a low pH. Similarly, to select for alkali-tolerant organisms, a high-pH medium can be used. For example, to select for the cholera-causing bacterium, *Vibrio cholerae*, from a stool sample, we can use a medium with a pH of 8.5; most other intestinal bacteria are unable to grow at this pH.

ell Size and Motility

We can sometimes make use of a small cell diameter or of bacterial motility to achieve selection. For instance, *Treponema* species from the human oral cavity can be selected by taking advantage of both of these properties. A membrane filter having a pore size of 0.15 μm is placed on the surface of an agar plate and gingival scrapings are placed on the filter. The unusually small size of treponemes allows them to penetrate the pores of the filter to reach the underlying agar. Moreover, treponemes have the ability to swim through solid agar media; consequently, they migrate away from the filter and grow to form a hazy zone within the agar, from which they can be subcultured. Other bacteria from the oral cavity are either too large to penetrate the membrane filter or, if they can penetrate it, are unable to migrate away through the agar.

Biological Methods of Selection

A disease-producing species occurring in a mixed culture can often be selected by taking advantage of its pathogenic properties. For example, a sputum sample containing *Streptococcus pneumoniae* is ordinarily contaminated by many other bacterial species. However, laboratory mice are extremely susceptible to infection by *S. pneumoniae*, and if the sputum sample is injected into a mouse the pathogen will multiply extensively. Nonpathogenic bacteria present in the sample will either be inhibited or killed by the defense mechanisms of the animal. In a sense, the animal serves as the selective medium.

How can the microbiologist know what selective media or conditions to use for a given species? Many selective methods are given in the references at the end of this chapter. Moreover, you can often devise a satisfactory selective procedure by comparing the characteristics of the species sought with those of the accompanying contaminants. Differences in these characteristics, e.g., in susceptibility to certain antibiotics, can provide the basis for a suitable selective procedure.

Selection in Nature

It is important to realize that the principle of selection is not limited to the laboratory; it also commonly operates in nature. For instance, the occurrence of high salt concentrations in bodies of water such as the Dead Sea selects for extreme halophiles such as those of the genus *Halobacterium*. In lakes, the anaerobic, sulfide-containing zone on or above the sediment mud provides conditions that often favor the mass development of green or purple sulfide-oxidizing, phototrophic bacteria. The nodules that occur on the roots of leguminous plants contain bacteria of the genus *Rhizobium*, which are uniquely suited for nitrogen fixation in association with these plants. In many types of natural infections of humans or animals, a single, uncontaminated pathogenic bacterial species can often be obtained from a blood sample; blood from a healthy animal or human is normally free of bacteria. Numerous other examples of selective conditions in nature exist.

PURE CULTURES

If the bacterial species being sought comprises a suitably high proportion of the mixed population, it can be isolated in pure culture. The descendents of a single isolation in pure culture comprise a **strain**. A strain is usually made up of a succession of cultures and is often derived from a single colony; however, the number of bacteria which gave rise to the original colony is usually unknown. If a strain is derived from a single parent cell, it is termed a **clone**. Each strain

is designated by an identifying number and its history is recorded (the source from which the isolation was made, the name of the person who made the isolation, the date of the isolation, and the culture collection in which the strain is maintained and from which it can be obtained for study).

A variety of techniques have been developed whereby isolation into pure culture can be accomplished. Each technique has certain advantages and limitations, and there is no one method that can be used for all bacteria.

Methods of Isolating Pure Cultures

The Streak-Plate Technique

By means of a transfer loop, a portion of the mixed culture is placed on the surface of an agar medium and streaked across the surface. This manipulation "thins out" the bacteria on the agar surface so that some individual bacteria are separated from each other. Figure 8-2 illustrates a nutrient-agar plate culture that has been streaked to provide isolated colonies. When streaking is properly performed, the bacterial cells will be sufficiently far apart in some areas of the plate to ensure that the colony developing from one cell will not merge with that growing from another. Figure 8-3 illustrates a modification known as the roll-tube technique that is used for the isolation of stringent anaerobes.

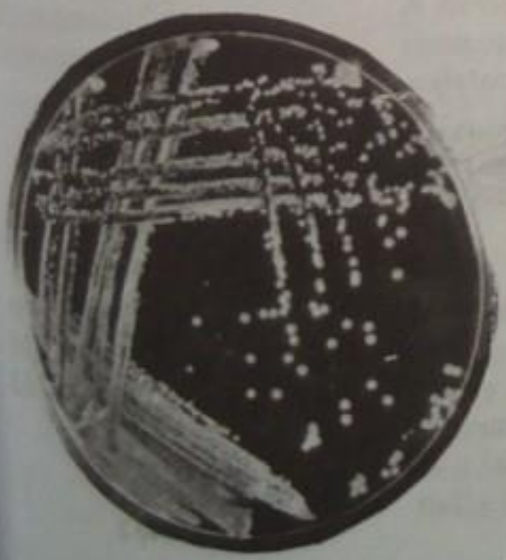

Figure 8-2. Streak-plate culture showing areas of isolated colonial growth. Note that where the colonies are sparse they are larger than when crowded together. (Courtesy of Naval Biological Laboratory.)

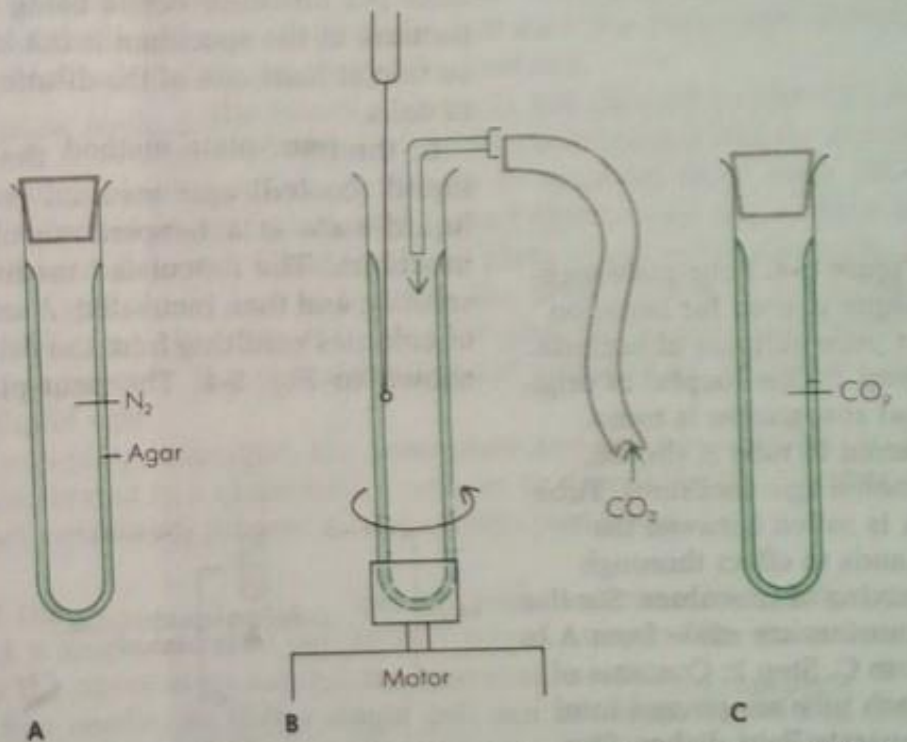

Figure 8-3. Roll-tube method for isolating stringent anaerobes. (A) Stoppered anaerobic culture tube whose inner walls have been coated with a prereduced agar medium. The tube contains an atmosphere of oxygen-free N_2. (B) When the stopper is removed the tube is kept anaerobic by continuously flushing it with oxygen-free CO_2 from a gas cannula. Inoculation is done with a transfer loop held against the agar surface as the tube is being rotated by a motor. By starting at the bottom and drawing the loop gradually upward, the inoculum becomes "thinned" to the point where well-isolated colonies can develop. (C) After inoculation the tube is restoppered and incubated.

The assumption is often made that a colony is derived from a single cell and, therefore, that the colony is a clone. However, this is not necessarily true. With species in which the cells form a characteristic grouping during cell division (for example, clumps of staphylococci or chains of streptococci), the colony may develop from a group of cells rather than from a single cell. Although not a clone, such a colony is nevertheless a pure culture if it contains only one kind of organism.

One should recognize that subculturing a colony from a single streak plate does not automatically assure purity. The colony may have been derived from two or more different kinds of bacteria. For example, when we attempt to isolate slime- or chain-producing bacteria, contaminants may be found to have adhered to the slime or to have been enmeshed in the network of chains, thereby resulting in impure colonies. The use of selective media can also lead to impure colonies. Although the growth of contaminants is inhibited on selective media, low numbers of viable cells may still be present, and such cells can be subcultured along with a colony. For these reasons, it is advisable to streak a culture several times in succession, preferably on nonselective media, in order to ensure purity.

The Pour-Plate and Spread-Plate Techniques

In both of these methods the mixed culture is first diluted to provide only a few cells per milliliter before being used to inoculate media. Since the number of bacteria in the specimen is not known beforehand, a *series of dilutions* is made so that at least one of the dilutions will contain a suitably sparse concentration of cells.

In the **pour-plate method** the mixed culture is diluted directly in tubes of liquid (cooled) agar medium (see Fig. 8-4). The medium is maintained in a liquid state at a temperature of 45°C to allow thorough distribution of the inoculum. The inoculated medium is dispensed into Petri dishes, allowed to solidify, and then incubated. A series of agar plates showing decreasing numbers of colonies resulting from the dilution procedure in the pour-plate technique is shown in Fig. 8-4. The pour-plate technique has certain disadvantages. For

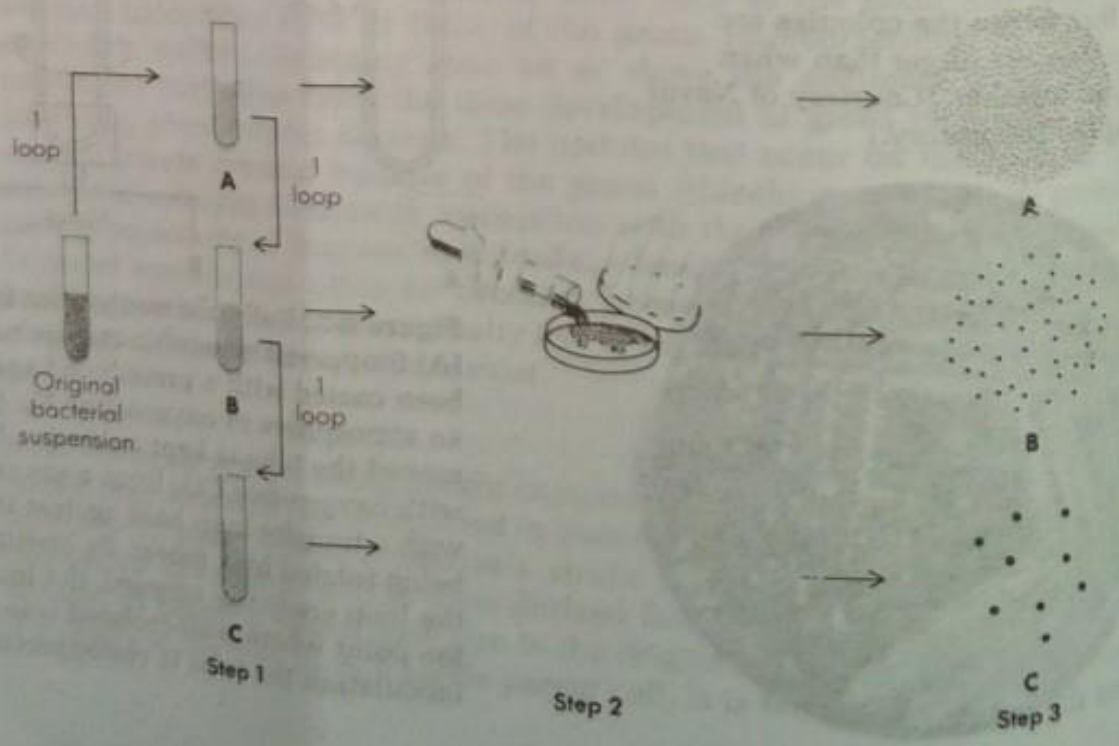

Figure 8-4. Pour-plate technique is used for isolation of pure cultures of bacteria. Step 1: One loopful of original suspension is transferred to tube A (liquid, cooled agar medium). Tube A is rolled between the hands to effect thorough mixing of inoculum. Similar transfers are made from A to B to C. Step 2: Contents of each tube are poured into separate Petri dishes. Step 3: After incubation, plates are examined for the one which contains well-separated colonies. From this plate, pure cultures of bacteria can be isolated by transferring a portion of a colony to a tube of sterile medium.

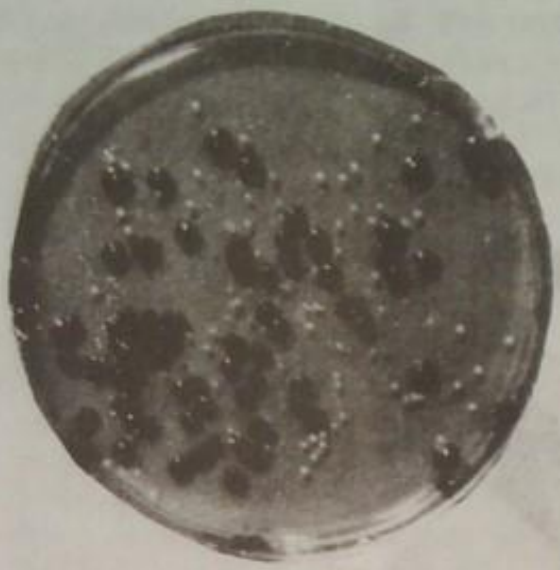

Figure 8-5. Spread plate showing colonies of two different bacterial species. A dilution of the mixed culture was spread over the surface with a glass rod. The large, dark colonies are *Serratia marcescens*, which has a brick-red pigment, and the smaller, light colonies are *Micrococcus luteus*, which has a lemon-yellow pigment. *(Courtesy of Naval Biological Laboratory.)*

instance, some of the organisms are trapped beneath the surface of the medium when it gels, and therefore both *surface* and *subsurface* colonies develop. The subsurface colonies can be transferred to fresh media only by first digging them out of the agar with a sterile instrument. Another disadvantage is that the organisms being isolated must be able to withstand temporary exposure to the 45°C temperature of the liquid agar medium; for instance, the pour-plate method would be unsuitable for isolating psychrophilic bacteria.

In the **spread-plate method** the mixed culture is not diluted in the culture medium; instead it is diluted in a series of tubes containing a sterile liquid, usually water or physiological saline. A sample is removed from each tube, placed onto the surface of an agar plate, and spread evenly over the surface by means of a sterile, bent glass rod. On at least one plate of the series the bacteria will be in numbers sufficiently low as to allow the development of well-separated colonies (see Fig. 8-5). In contrast to the pour-plate technique, only surface colonies develop; moreover, the organisms are not required to withstand the temperature of liquid agar.

Unlike the streak-plate technique, the pour-plate and the spread-plate techniques may be performed in a *quantitative* manner to determine the number of bacteria (of a particular type) present in a specimen (see Chap. 7).

nipulator
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A device called the **micromanipulator** can be used in conjunction with a microscope to pick a single bacterial cell from a mixed culture. The micromanipulator permits the operator to control the movements of a **micropipette** or a **microprobe** (a fine needle) so that a single cell can be isolated (see Fig. 8-6). This technique requires a skilled operator and is reserved for studies in which a clone must be obtained unequivocally.

ENANCE AND
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Most microbiology laboratories maintain a large collection of strains, frequently referred to as a **stock-culture collection.** These organisms are needed for laboratory classes and research work, as test agents for particular procedures, or as reference strains for taxonomic studies. Most major biological companies maintain large culture collections. The strains are used for screening of new, potentially effective chemotherapeutic agents; as assay tools for vitamins and amino

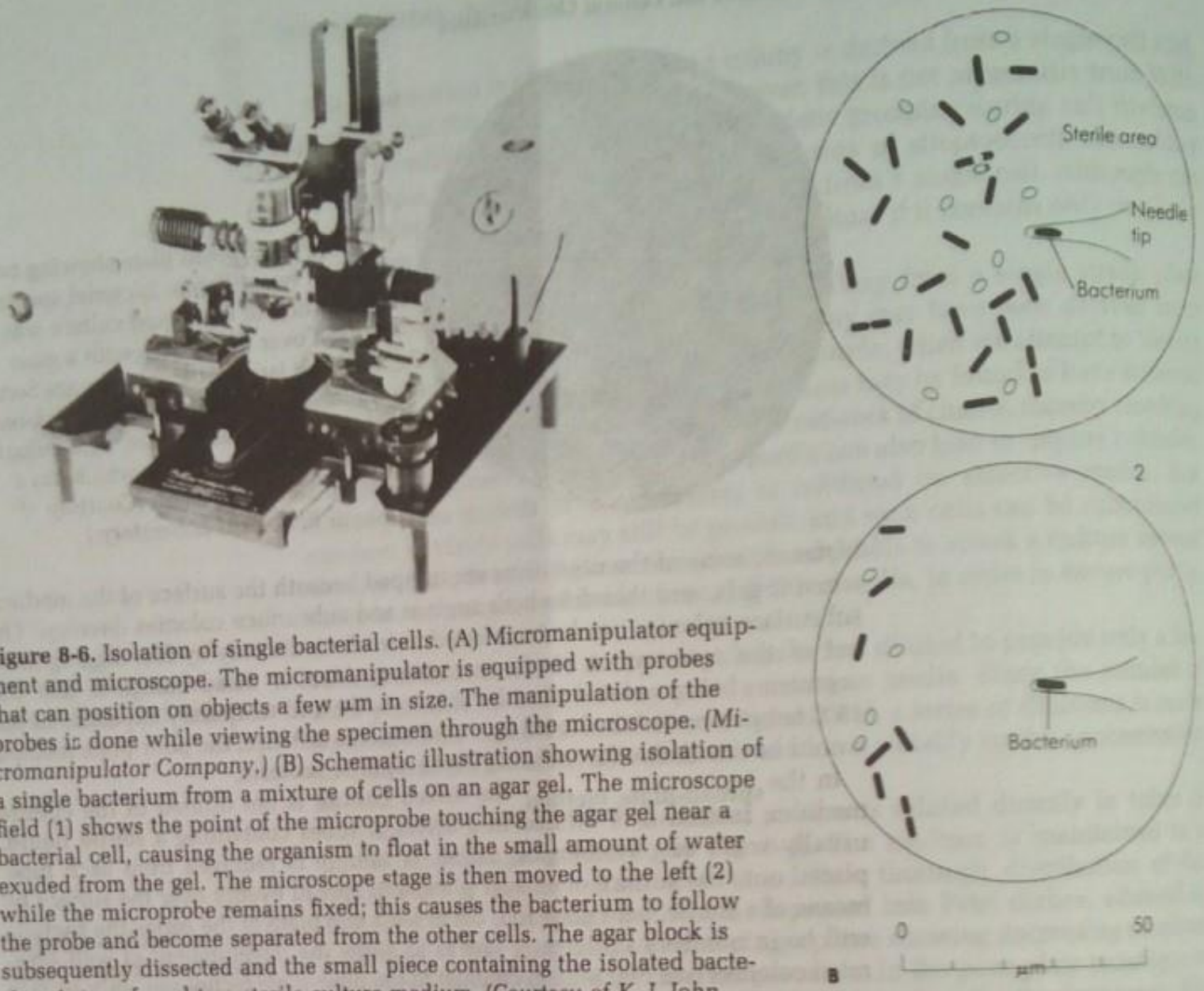


Figure 8-6. Isolation of single bacterial cells. (A) Micromanipulator equipment and microscope. The micromanipulator is equipped with probes that can position on objects a few μm in size. The manipulation of the probes is done while viewing the specimen through the microscope. *(Micromanipulator Company.)* (B) Schematic illustration showing isolation of a single bacterium from a mixture of cells on an agar gel. The microscope field (1) shows the point of the microprobe touching the agar gel near a bacterial cell, causing the organism to float in the small amount of water exuded from the gel. The microscope stage is then moved to the left (2) while the microprobe remains fixed; this causes the bacterium to follow the probe and become separated from the other cells. The agar block is subsequently dissected and the small piece containing the isolated bacterium is transferred to a sterile culture medium. *(Courtesy of K. I. Johnstone, Manipulation of Bacteria, Churchill Livingstone, Edinburgh, 1973.)*

acids; as agents for the production of vaccines, antisera, antitumor agents, enzymes, and organic chemicals; and as reference cultures that are cited in company patents. For these and other purposes it is extremely important to have properly identified and cataloged strains of bacteria available. Consequently, a considerable amount of research has been performed to develop methods whereby bacterial strains can be preserved and stored until they are needed. Several different methods have been developed, since not all bacteria respond in a similar manner to a specific method. Moreover, there are various practical considerations such as the amount of labor involved and the amount of storage space required. However, all the methods which we will now describe have the same objective: to maintain strains alive and uncontaminated and to prevent any change in their characteristics.

Methods of Maintenance and Preservation

Strains can be maintained by periodically preparing a fresh stock culture from the previous stock culture. The culture medium, the storage temperature, and the time interval at which the transfers are made vary with the species and must

dic Transfer to Media

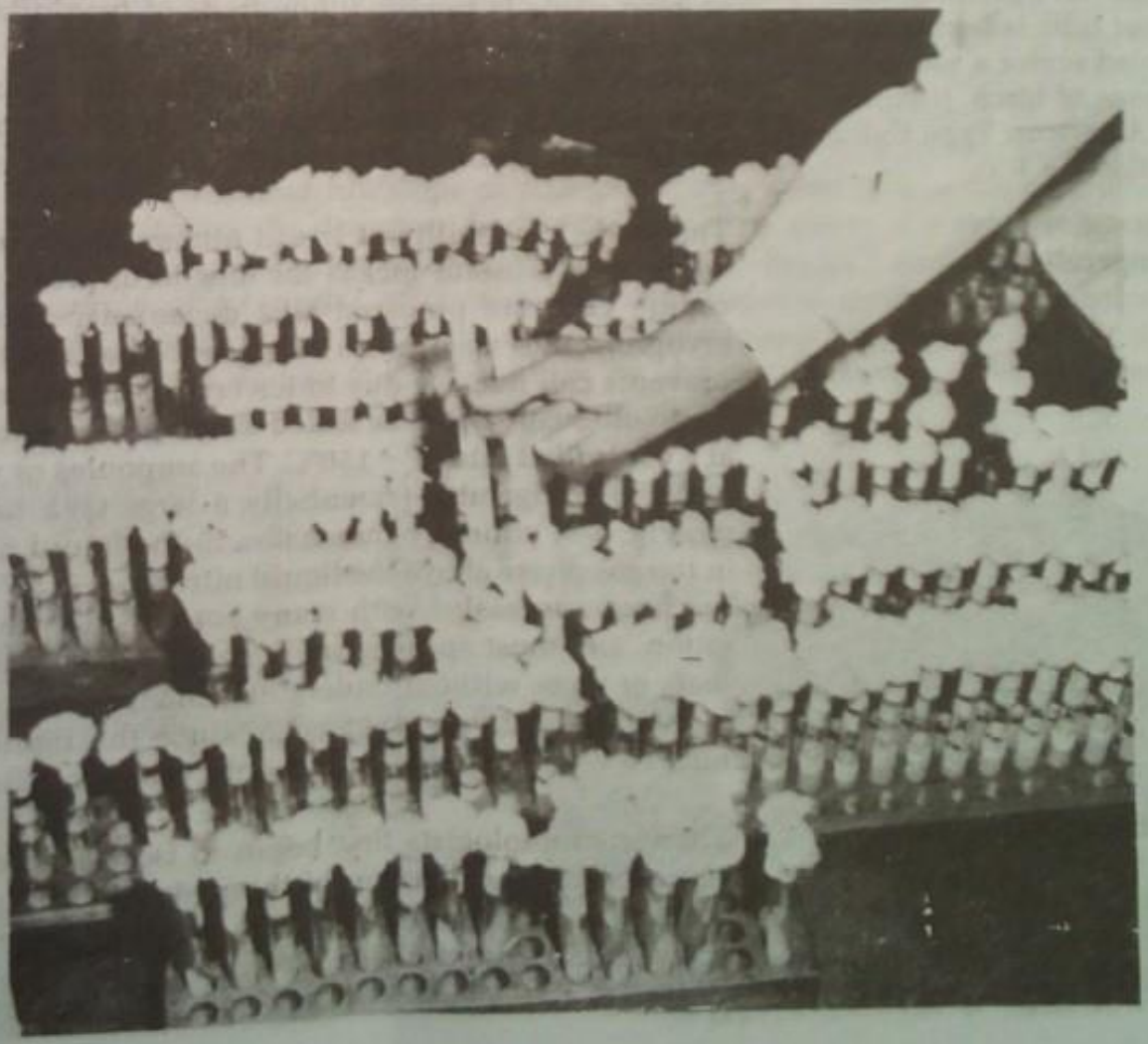
be ascertained beforehand. The temperature and the type of medium chosen should support a slow rather than a rapid rate of growth so that the time interval between transfers can be as long as possible. Many of the more common heterotrophs remain viable for several weeks or months on a medium like nutrient agar. The transfer method has the disadvantage of failing to prevent changes in the characteristics of a strain due to the development of variants and mutants.

ervation by Overlaying ures with Mineral Oil

Many bacteria can be successfully preserved by covering the growth on an agar slant with sterile mineral oil. The oil must cover the slant completely; to ensure this, the oil should be about ½ in above the tip of the slanted surface. Maintenance of viability under this treatment varies with the species (1 month to 2 years). This method of maintenance has the unique advantage that you can remove some of the growth under the oil with a transfer needle, inoculate a fresh medium, and still preserve the original culture. The simplicity of the method makes it attractive, but changes in the characteristics of a strain can still occur. Figure 8-7 illustrates a culture collection maintained by this technique.

servation by Lyophilion (Freeze-Drying)

Most bacteria die if cultures are allowed to become dry, although spore- and cyst-formers can remain viable for many years. However, freeze-drying can satisfactorily preserve many kinds of bacteria that would be killed by ordinary



ure 8-7. A culture collection maintained by overlaying cultures with mineral . *(Courtesy of U.S. Department of Agriculture.)*

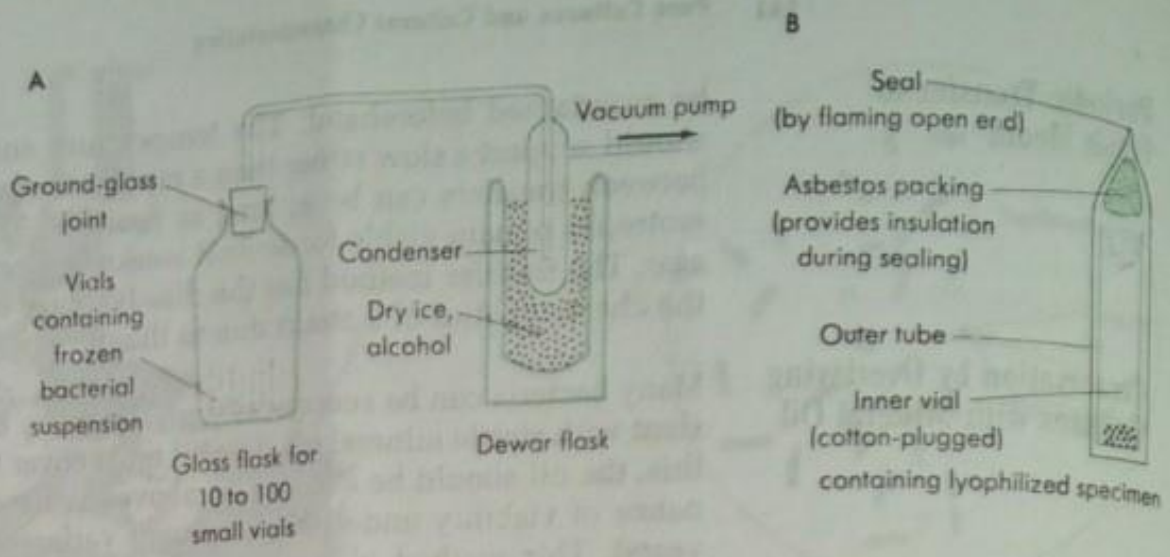


Figure 8-8. Lyophilization process for preservation of cultures. (A) A simple apparatus for lyophilization. Small cotton-plugged vials containing frozen suspensions of bacteria are placed in the glass flask, which is attached to a condenser. The condenser is connected to a high-vacuum pump. The bacteria become desiccated as the ice in the frozen suspension sublimes directly to water vapor. The vapor is trapped on the cold surface of the condenser, thereby preventing it from entering the vacuum line and contaminating the pump oil. (B) After desiccation of the cultures as in (A), the vials are removed and each is placed in a larger tube. After insulating the vial with a plug of glass wool packing, the outer tube is hermetically sealed under a vacuum by means of torch. *(Courtesy of American Type Culture Collection.)*

drying. In this process a dense cell suspension is placed in small vials and frozen at −60 to −78°C. The vials are then connected to a high-vacuum line. The ice present in the frozen suspension sublimes under the vacuum, i.e., evaporates without first going through a liquid water phase. This results in dehydration of the bacteria with a minimum of damage to delicate cell structures. The vials are then sealed off under a vacuum and stored in a refrigerator. One arrangement of equipment employed to lyophilize cultures is shown in Fig. 8-8. Many species of bacteria preserved by this method have remained viable and unchanged in their characteristics for more than 30 years. Only minimal storage space is required; hundreds of lyophilized cultures can be stored in a small area. Furthermore, the small vials can be sent conveniently through the mail to other microbiology laboratories when packaged in special sealed mailing containers. Lyophilized cultures are revived by opening the vials, adding liquid medium, and transferring the rehydrated culture to a suitable growth medium.

Storage at Low Temperatures

The ready availability of liquid nitrogen has provided the microbiologist with another very useful means for long-term preservation of cultures. In this procedure cells are prepared as a dense suspension in a medium containing a cryoprotective agent such as glycerol or dimethyl sulfoxide (DMSO), which prevents cell damage due to ice crystal formation during the subsequent steps. The cell suspension is sealed into small ampoules or vials and then frozen at a controlled rate to −150°C. The ampoules or vials are then stored in a liquid nitrogen refrigerator (essentially a large tank having vacuum-insulated walls; see Fig. 8-9) either by immersion in the liquid nitrogen (−196°C) or by storage in the gas phase above the liquid nitrogen (−150°C). The liquid nitrogen method has been successful with many species that cannot be preserved by lyophilization, and most species can remain viable under these conditions for 10 to 30 years or more without undergoing change in their characteristics. However, the method is relatively expensive, since the liquid nitrogen in the refrigerators must be replenished at regular intervals to replace the loss due to evaporation.

Culture Collections

When microbiologists first began to isolate pure cultures, each microbiologist kept a personal collection of those strains having special interest. Subcultures of some strains were often sent to other microbiologists; other subcultures were received and added to the scientist's own collection. Certain strains had tax-

onomic importance because they formed the basis for descriptions of species and genera. Others had special properties useful for various purposes. However, many important strains became lost or were inadequately maintained. Thus, it became imperative to establish large central collections whose main purpose would be the acquisition, preservation, and distribution of authentic cultures of living microorganisms.

Many countries have at least one central collection. As examples, in France a collection of bacteria is maintained at the Institut Pasteur in Paris; in England the National Collection of Type Cultures is in London; the Federal Republic of Germany maintains the Deutsche Sammlung von Mikroorganismen in Darmstadt; and Japan maintains a large collection at the Institute for Fermentation in Osaka. Many other such collections exist.

In the United States the major collection is the American Type Culture Collection (ATCC), located in Rockville, Maryland. In 1980 the collection included the following numbers of strains: bacteria, 11,500; bacteriophages, 300; fungi and fungal viruses, 13,700; protozoa, 720; algae, 130; animal-cell cultures, 500; animal viruses, rickettsiae, and chlamydiae, 1,135; and plant viruses, 220. More than 1 million ampoules of lyophilized or frozen living strains are inventoried and stored at the ATCC. Other large collections in the United States are more specialized in scope. For example, the Northern Utilization Research and Development Division, USDA, at Peoria, Illinois, maintains a collection of yeasts, molds, and bacteria especially for use in fermentations. The Quartermaster Research and Development Center, U.S. Army, Natick, Massachusetts, maintains a collection of microbial strains that are associated with deterioration processes. A number of smaller collections of a specialized nature also exist, such as the collection of anaerobic bacteria maintained by the Department of Anaerobic Microbiology, Virginia Polytechnic Institute and State University, Blacksburg.

One of the major functions of a large national collection is the preservation of type strains. As discussed in Chap. 3, the type strain of a species has great taxonomic importance because it is the "name-bearer" strain, or permanent example, of the species. Microbiologists who propose a new species are expected to deposit the type strain with one or more national collections so that it can be preserved and so that subcultures can be distributed to other workers for study and comparison with other microorganisms.

Figure 8-9. (A) Liquid-nitrogen refrigerators used for preservation of bacteria. Each container holds many thousands of ampoules. *(Courtesy of American Type Culture Collection.)* (B) Preservation of bacterial cultures in the gas phase (−150°C) of liquid-nitrogen refrigerators. For preservation, a bacterial suspension is placed in a vial which is then sealed. This picture shows six vials, attached to a metal cane, being removed from storage. *(Courtesy of Alma Dietz, The Upjohn Company.)*

CULTURAL CHARACTERISTICS

One of the major features of a bacterial strain is its appearance following growth on various media. Such commonplace characteristics as the abundance of the growth, the size of the colonies, and the color (or chromogenesis) of the colonies provide useful clues for identification.

To determine the growth characteristics of a bacterial strain, it is customary to observe the features of colonies and broth cultures. Inoculation of agar plates to obtain isolated colonies has already been described. Tubes of broth can be inoculated with the transfer needle or the loop; generally the loop is used when the inoculum is a liquid.

After inoculation of the medium and subsequent incubation, the cultural characteristics can be determined. The main features can be summarized as follows.

Colony Characteristics

Size. Colonies range in size from extremely small (pinpoint), measuring only a fraction of a millimeter in diameter, to large colonies measuring 5 to 10 mm in diameter. Although the colonies of a given species have a characteristic diameter, one must be aware of certain factors affecting colony diameter. For instance, only well-separated colonies should be measured, since such colonies tend to have a larger diameter than those which are crowded together (for example, see Fig. 8-2). This is because widely separated colonies are subject to less competition for nutrients and less inhibition by toxic products of metabolism. Moreover, young colonies are smaller than older colonies; therefore the time at which measurements are made must be stated. There is generally an upper limit to the final size of the colonies of a given species; i.e., a point is reached where further incubation no longer results in a corresponding increase in size. However, some bacteria (e.g., certain species of *Proteus* and *Bacillus*) can spread across the entire agar surface, and the colony size is limited only by the dimensions of the Petri dish!

Margin or Edge. The periphery of bacterial colonies may take one of several different patterns, depending on the species. It may be evenly circular like the edge of a droplet or it may show irregularities such as rounded projections, notches, and threadlike or rootlike projections.

Surface Texture. Depending on the species, the colony surface may be smooth (shiny, glistening); rough (dull, granular, or matte); or mucoid (slimy or gummy). Certain species have colonies posessing a highly wrinkled surface.

For a pure culture, all the colonies on the plate should have a similar type of surface; however, you should bear in mind that some pure cultures may exhibit surface variation. One of the commonest variations is known as the S → R variation. This is due to the presence of mutant cells that give rise to some rough (R) colonies in a population that otherwise produces smooth (S) colonies. Some R mutants produce rough colonies because they lack the ability to make capsules, or, if the species is Gram-negative, they may no longer be able to form O antigens.

For several species of pathogenic bacteria, the surface texture of colonies may bear a relation to virulence. For instance, S colonies of *S. pneumoniae* or of *Salmonella* species are usually virulent, whereas R colonies are not. On the other hand, for strains of *Mycobacterium tuberculosis* a rough surface showing serpentine cords is usually a good indicator of virulence.

Elevation. Depending on the species, colonies may be thin to thick, and the surface may be flat or it may exhibit varying degrees of convexity.

Consistency. This can be determined by touching a transfer needle to the colony. Some bacterial species form colonies having a butyrous or butterlike consistency. Others may form colonies that are viscous, stringy, or rubbery; in the latter type the whole colony, rather than just a portion of it, may come off the agar surface with the transfer needle. Still other species may form dry, brittle, or powdery colonies that break up when touched with the needle.

Optical features. Colonies may be opaque, translucent, or opalescent.

Chromogenesis or Pigmentation. Some bacterial species produce and retain water-insoluble pigments intracellularly, thus causing the colonies to become colored (pigmented). Some species which form pigmented colonies are:

Flectobacillus major	Pink
Serratia marcescens	Red
Chromobacterium violaceum	Violet
Staphylococcus aureus	Gold
Micrococcus luteus	Yellow
Derxia gummosa	Brown
Bacteroides melaninogenicus	Black

Some colonies produce pigments that are water-soluble; these diffuse into the surrounding agar and stain it. For instance, *Pseudomonas aeruginosa* forms a blue water-soluble pigment called pyocyanin. Some pigments are only sparingly water-soluble and may precipitate in the medium. For example, *Pseudomonas chlororaphis* forms a pigment called chlororaphin which accumulates in the form of green crystals around the colonies.

Certain water-soluble pigments are fluorescent; i.e., the agar medium around the colonies glows white or blue-green when exposed to ultraviolet light. For example, *P. aeruginosa* produces not only the nonfluorescent pigment pyocyanin but also a fluorescent pigment, pyoverdin.

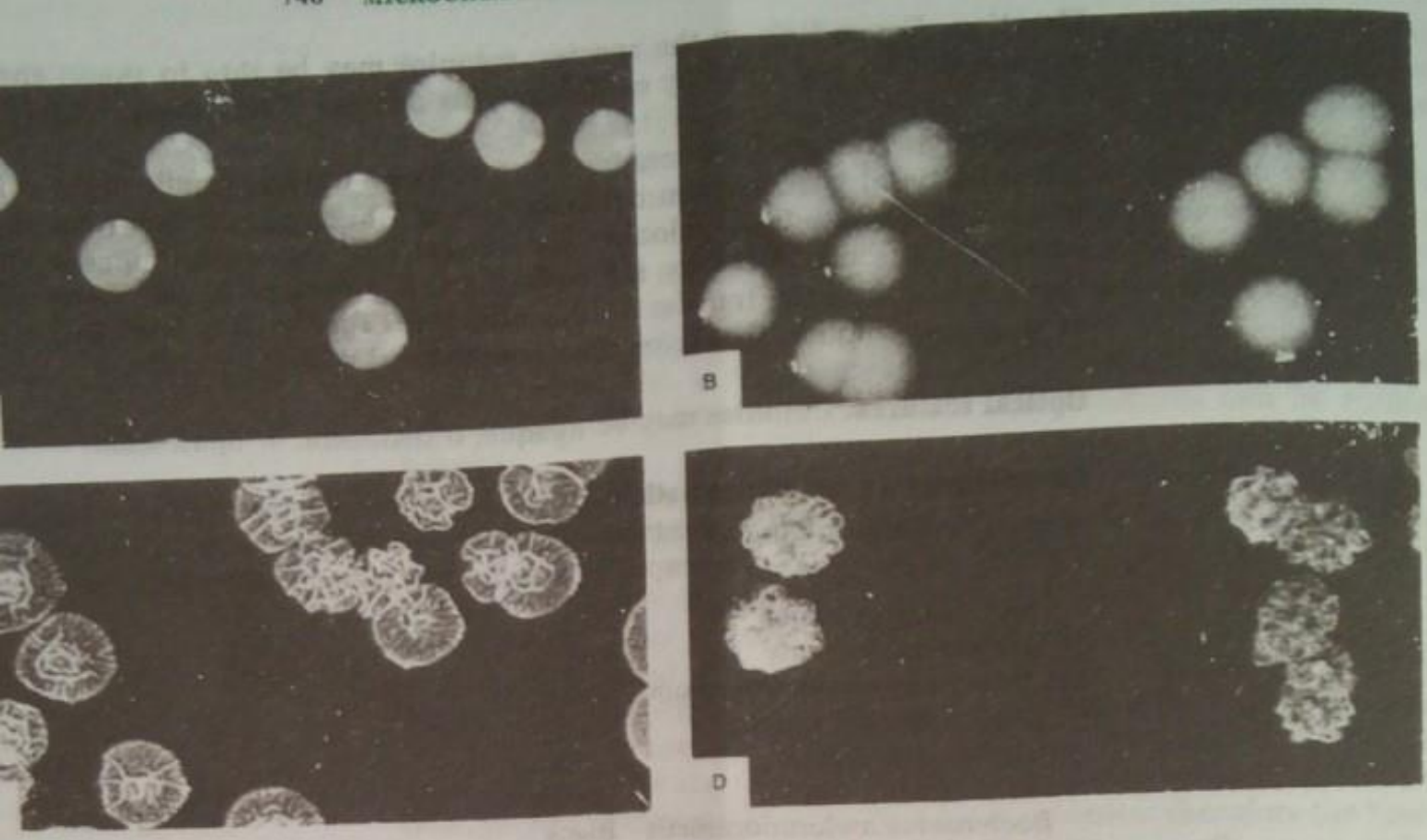
For a bacterial strain to exhibit its characteristic pigmentation, special media, incubation temperatures, or other conditions may be required. For instance, *Mycobacterium kansasii* forms a characteristic yellow pigment (β-carotene) only when the colonies are exposed to light.

Several types of bacterial colonies are shown in Fig. 8-10.

racteristics of
h Cultures

1 ***Amount of growth.*** Scanty, moderate, or abundant.
2 ***Distribution and type of growth.*** The growth may be uniformly distributed throughout the medium (evenly turbid). Alternatively, it may be confined to the surface of the broth as a scum or film (pellicle), or it may accumulate as a sediment, which may be granular or viscous.

The scheme for interpreting the appearance of bacterial growth has been described in some detail to emphasize the fact that many differences in cultural characteristics do occur among bacteria. With experience, familiarity with such characteristics becomes very helpful as a guide for the recognition of major groups of bacteria. Too often students pay little attention to these features of



ure 8-10. Bacterial colo-
s illustrating differences
characteristics. (A) Circu-
, raised, smooth surface;
circular, raised, finely
nular surface; (C) irregu-
edge, flat, elevated folds
surface; (D) undulate
ge, raised, irregularly ele-
ed surface. *(Courtesy of*
val Biological Labora-
y.)

bacterial growth and thus deprive themselves of much useful information in the laboratory study of cultures.

To further emphasize the importance of cultural characteristics, suppose that we have prepared plate and broth cultures of an unidentified strain designated as strain 24. The colonies are irregular and raised and appear dry, with a roughened, granular surface. When we touch a colony with a transfer needle it proves to be brittle, and when a portion of the colony is removed it will not emulsify easily when spread in a drop of water. Growth in broth cultures occurs mainly in the form of a heavy surface pellicle, and the medium below the pellicle is only slightly turbid. Familiarity with the cultural appearance of bacteria would suggest that strain 24 might be an acid-fact bacterium (*Mycobacterium*). Additional tests must be performed to verify this possibility, but the cultural characteristics have provided a clue to the type of organism we are working with.

ESTIONS

1 Devise an enrichment procedure for an aerobic bacterial species that can use methane gas as a sole carbon and energy source.

2 During an epidemic of meningitis caused by *Neisseria meningitidis*, many people become healthy carriers (i.e., harbor the organism in their nasopharynx but do not have meningitis) and can spread the organism by coughing and sneezing. Suppose you are given the task of determining how many people in the epidemic region are healthy carriers. You soon discover that the human nasopharynx is inhabited by many different kinds of microorganisms. However, you learn from selected references that *N. meningitidis*

is resistant to the chemotherapeutic agents vancomycin, colistin, and nystatin but is susceptible to penicillin. On the basis of this information devise a selective medium that could help you in your task.

3 What selective procedure would you use in the process of isolating from a soil sample (*a*) an endospore-forming organism, (*b*) a nonsporeforming gliding organism, (*c*) a psychrophilic organism?

4 Give three examples of selective mechanisms occurring in nature that lead to the predominance of particular kinds of bacteria.

5 Distinguish between the meanings of the terms mixed culture, pure culture, clone, and strain.

6 Compare the advantages and disadvantages of the various techniques for the isolation of microorganisms in pure culture.

7 What are the advantages and disadvantages of the various methods for preservation of pure cultures?

8 Why have organizations been established to maintain pure cultures? Of what use are such collections?

9 What difficulty might exist in subculturing the colony of a desired organism from a selective agar medium? What additional steps should be taken to help assure culture purity?

10 How could you acquire a subculture of the type strain of *Streptococcus lactis* so that you could compare its characteristics with another strain that you have isolated from milk?

11 What general categories of pigments are produced by bacteria? For each category give an example of an organism that makes such a pigment.

12 Give several reasons why industrial biological companies maintain large stock-culture collections.

American Type Culture Collection: *Catalog of Strains*, vol. 1, 15th ed., American Type Culture Collection, Rockville, Md., 1982. *Contains a listing of the extensive holdings of the collection for algae, bacteria, bacteriophages, fungi and fungal viruses, plant viruses, protozoa, plasmids, and recombinant DNA vectors. References, conditions for cultivation, media formulations, and other information are provided.*

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tive methods are given for various species of bacteria. Also presented are the details of the methods for isolating bacteria into pure culture.

Krieg, N. R. (ed.): Bergey's Manual of Systematic Bacteriology, vol. 1, Williams & Wilkins, Baltimore, 1984. This together with subsequent volumes of this international reference work provides the characteristics of the genera and species of bacteria, including methods for the selection, isolation, and maintenance of each group.

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