Isolation and Characterization of Soil Bacteria: A Review

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Abstract
Soil is a dynamic ecosystem teeming with microbial life that plays a vital role in nutrient cycling, organic matter decomposition, and overall soil fertility. Among the various microorganisms, bacteria are the most abundant and functionally diverse group, contributing significantly to ecological balance and biogeochemical transformations. Understanding soil bacterial diversity requires their isolation, cultivation, and characterization under laboratory conditions. This review consolidates existing knowledge on methods of bacterial isolation from soil, cultural and morphological characterization, and biochemical profiling techniques. Special emphasis is given to conventional and modern isolation methods, staining procedures, and the taxonomic importance of bacterial identification. The study further highlights the ecological and applied significance of soil bacteria in biodegradation, bioremediation, and soil health maintenance.

Keywords: Soil microbiology, bacterial isolation, characterization, pure culture, staining, colony, morphology.

Introduction

Bacteria, the most abundant and metabolically diverse organisms in soil ecosystems, drive nutrient cycling, organic matter decomposition, nitrogen fixation, and the transformation of pollutants, thereby maintaining soil fertility and ecological balance (Alexander, 1997). Soil bacterial communities are profoundly influenced by environmental parameters such as soil pH, moisture, aeration, and organic matter content (Killham, 1994). This review consolidates both classical and recent findings related to the isolation and characterization of soil bacteria, emphasizing methodological rigor, ecological insight, and future research directions. Bacteria are among the earliest and most successful forms of life on Earth, exhibiting extraordinary adaptability and ecological importance. They are single-celled, prokaryotic organisms lacking a defined nucleus, and generally range from 0.5 to 2.0 µm in diameter. Bacterial morphology varies widely, with common forms including cocci (spherical), bacilli (rod-shaped), and spirilla (spiral). Bacteria are the most abundant and metabolically diverse organisms in soil ecosystems, representing up to 10^9 cells per gram of soil. Their metabolic diversity makes them indispensable in nutrient cycling and the maintenance of soil structure. Furthermore, bacteria are instrumental in bioremediation processes, such as the degradation of hydrocarbons and other organic pollutants (Brady and Weil, 2002). In soil ecosystems, bacteria serve as decomposers, nitrogen fixers, and agents of organic matter transformation (Stevenson, 1986). A series of studies conducted by Chowdhury (2020,

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2022) systematically characterized bacterial communities across different soil series of Bangladesh, Amjhupi, Ishurdi, Sonatala, Bajoa demonstrating the heterogeneity of bacterial populations and their adaptive characteristics. These works have contributed significantly to understanding regional soil microbiology. Understanding bacterial diversity and function requires accurate isolation and identification. The classification of soil bacteria enables researchers to explore their ecological roles and potential applications in agriculture, biotechnology, and medicine (Joklik et al., 1992). However, despite their ubiquity, research on soil microbiology—particularly in developing regions such as Bangladesh—remains limited. Systematic studies on bacterial isolation and characterization can therefore provide essential insights into soil ecosystem functioning.

Literature Review

Soil Bacteria

Bacteria are the most prevalent microbial inhabitants of soil, though their total biomass is generally lower than that of fungi. Soil bacterial cells are typically small—about 1–2 µm in length and 0.5–1 µm in diameter—and exhibit diverse morphologies, such as bacilli (rod-shaped), cocci (spherical), spirilla (helical), or vibrios (S-shaped). These bacteria can be motile or non-motile, and are usually adsorbed onto soil particles through ionic interactions, often involving polyvalent cations that bridge negatively charged bacterial and soil surfaces. Most soil bacteria inhabit micropores (<10 µm) within soil aggregates, where they are protected from predators like protozoa and benefit from stable moisture conditions. Bacteria in soil occur either singly or in microcolonies. Evidence suggests that microbial composition varies within soil aggregates—Gram-negative bacteria and actinomycetes are more common within aggregates, while Gram-positive bacteria dominate in inter-aggregate spaces (Hattori, 1973; Killham, 1994).

Occurrence of Soil Bacteria

Bacteria are the smallest and most abundant soil microorganisms, playing vital roles in nutrient cycling, particularly in the carbon and nitrogen cycles. Despite their microscopic size, they exhibit significant ecological adaptability and interact closely with plants and other microorganisms (Pankhurst, 1978). Most soil bacteria are saprophytic or parasitic, relying on organic matter as they generally lack chlorophyll. They are unicellular, prokaryotic organisms, usually 0.5–1 µm wide and 1–10 µm long, with rod-shaped forms being dominant (Mishra, 2000). Many possess flagella for locomotion and reproduce mainly through binary fission under favorable conditions. Bacterial populations in soil range from 10^8 to 3×10^9 cells per gram of soil (Biswas & Mukherjee, 1997), although this varies with soil fertility and organic matter input. Populations tend to increase sharply following organic enrichment but stabilize over time as resources become limited (Mishra, 2000).

Concept of Bacterial Isolation

To study the properties of a specific bacterial species, researchers must obtain a pure culture—a population derived from a single cell type. The process of separating one microbial species from a mixed population is known as isolation (Benson, 2002).

Steps in the Isolation Process

Preparation of Sample

The bacterial source can be solid (e.g., soil) or liquid (e.g., water). For soil samples, bacteria are extracted using physiological saline (distilled water + 0.9% NaCl) to release microorganisms into suspension,

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while water samples can be used directly (Dubey & Maheshwari, 1999).

Preparation of Culture Media

The growth and survival of bacteria depend on nutrient availability and suitable environmental conditions. In laboratory studies, microorganisms are cultivated on media, which can be liquid, semisolid, or solid, depending on the presence of agar as a solidifying agent.

- Liquid media (e.g., nutrient broth, tryptic soy broth) are used for large-scale propagation and biochemical testing.
- Semisolid media are useful for motility and anaerobic growth studies.
- Solid media (e.g., nutrient agar, blood agar) support surface growth for colony observation, isolation, storage, and biochemical assays.

Solid media can be prepared as agar slants, agar deeps, or agar plates, depending on how they are cooled and solidified **(**Fig. 1). Media are further classified as chemically defined (synthetic)—containing known quantities of pure compounds—or complex (nonsynthetic), which include nutrient-rich natural ingredients like peptones or extracts. The latter are commonly used for culturing heterotrophic bacteria in laboratory practice (Prescott & Harley, 2002).

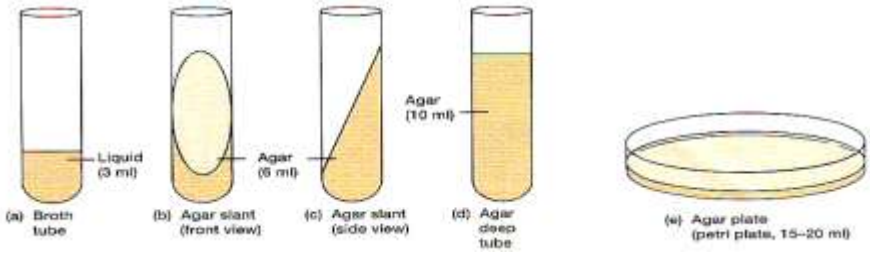

Fig. 1. Different forms of culture media (Prescott and Harley, 2002).

Sterilization of nutrient agar media and equipments used in bacterial culture

Sterilization is the process of rendering a medium or material free of all forms of life. There are three basic ways in which sterilization of media is achieved. The most useful approach is autoclaving, in which items are sterilized by exposure to steam at 121^0c and 15 lbs (pound per square inch) of pressure for 20 minutes or more, depending on the nature of the item. Under these conditions, microorganisms, even their endospores will not survive longer than about 15 to 20 minutes (Prescott and Harley, 2002).

Preparation of petriplate for bacterial culture

Glass test-tubes and glass or plastic petridishes are used to cultivate microorganisms. A suitable nutrient medium in the form of broth or agar may be added to the tubes. The media will supply the nutrition. The lid and base part of the petriplate provide a contamination free environment for the bacterial culture (Joklik et al., 1992).

Serial dilution of sample

Serial dilution is a process where original inoculum is diluted several times. It is important that only a limited number of colonies develop in the plate when too many colonies are present, some cells are overlapped which cannot be identified accurately. To ensure the distinct and separated colony, the original sample is diluted in a series of dilution tubes to reduce the concentration of bacterial population per unit of sample (Joklik et al., 1992).

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Inoculation of pure culture

There are several ways to prepare pure culture. Some of which are: 1) Use of micromanipulator, 2) Isolation by exposure to air, 3) Isolation by inoculating in animals, 4) Isolation by using selective or enrichment media, 5) Isolation by streak plate technique, 6) Isolation by spread plate technique, 7) Isolation by pour plate technique (Dubey and Maheshwari, 1999).

Spread plate technique

With the spread plate method**,** a volume of an appropriately diluted Culture usually no greater than 0.1 ml is spread over the surface of an agar plate using a sterile glass spreader **(**Fig. 2). The plate is then incubated until the colonies appear, and the number of colonies is counted. It is important that the Surface Of the plate be dry so that the liquid that is spread soaks in (Prescott and Harley, 2002).

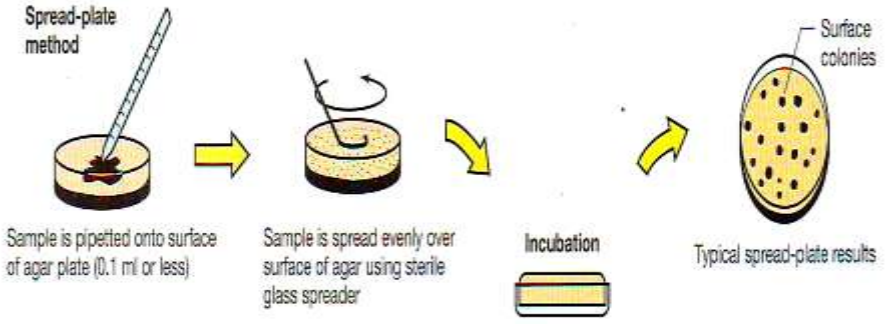

Fig. 2. The spread plate technique (Madigan *et al*., 2000).

Streak plate technique

It is a direct way of achieving the isolated pure colonies. In this technique, the bacterial mixture is transferred to the edge of an agar plate with an inoculating loop and then streaked out over the surface in one of several patterns **(**Fig. 3). At some point on the streaks, individual cells will be removed from the loop as it glides along the agar surface and will give rise to separate colonies. Again, one assumes that one colony comes from one cell. The key principle of this method is that by streaking, a dilution gradient is established on the surface of the plate as cells are deposited on the agar surface. Because of this gradient, confluent growth occurs on part of the plate where the cells are not sufficiently separated, and individual, well-isolated colonies develop in other regions of the plate where few enough cells are deposited to form separate colonies that can be seen with the naked eye. Cells from the new colony can then be picked up with an inoculating needle and transferred to an agar slant or other suitable medium for maintenance of the pure culture (Prescott and Harley, 2002).

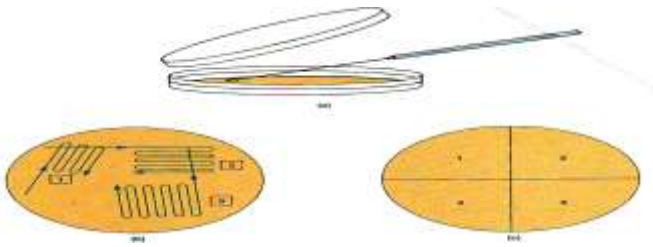

Fig. 3. The streak-plate technique (Prescott and Harley, 2002).

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Viable count

In the method just described, both living and dead cells are counted **(**Fig. 4). In many cases we are interested in counting only live cells, and for this purpose viable cell counting methods have been developed. A viable cell is defined as one that is able to divide and form offspring, and the usual way to perform a viable Count is to determine the number of cells in the sample capable of forming colonies on a suitable agar medium. For this reason, the viable count is often called the plate count, or colony count. There are two ways of performing a plate count: the spread plate method and the pour plate method (Madigan et al., 2000).

The final plates in the series should have between 25 and 250 colonies. Fewer than 25 colonies are not acceptable for statistical reasons, and more than 250 colonies on a plate are likely to produce colonies too close to each other to be distinguished as distinct colony-forming units (CFUs). The assumption is that each viable bacterial cell is separate from all others and will develop into a single discrete colony (CFU). Thus, the number of colonies should give the number of live bacteria that can grow under the incubation conditions employed (Prescott and Harley, 2002). Bacterial colonies grow from a single cell and are composed of millions of cells. Bacterial colonies grow from a single cell and are composed of millions of cells (Goodffllow and O'donnell, 1993).

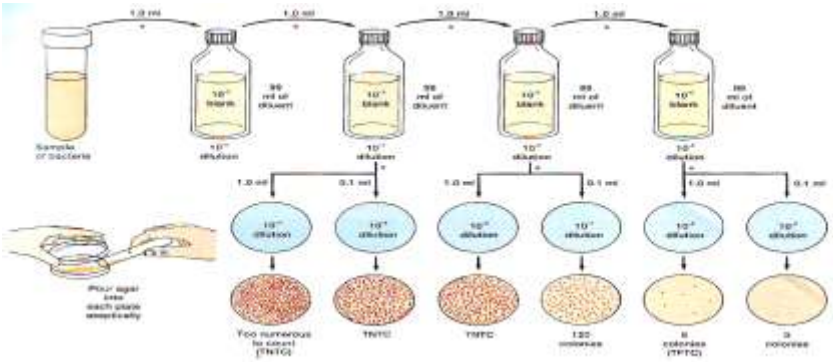

Fig. 4. Quantitative plating technique (Prescott and Harley, 2002).

Characterization

More scientific and systematic identification and classification of bacteria, three different characteristics have to must study. Without any of these three characteristic bacteria can not be identified. These characteristics are:

- Colonial characteristics
- Morphological characteristics
- Biochemical characteristics

Colony characteristics

A colony of bacteria refers to a group of bacteria formed by the reproduction of a single bacterial cell and the colony is visible to the naked eye. The colonies may be varying in diameter, in outline (circular wavy, rhizoid, etc) elevation (flat, raised, convex, etc.) and translucency (transparent, translucent and opaque). The bacterial colony may be colorless or white and certain chromomeric bacteria form colonies of

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various color. In some bacteria the background (medium) may get characteristic color. In liquid media, the bacteria may grow in abundance or could be sparse; they may be evenly dispersed throughout the medium or may occur only as sediment at the bottom or thin film at the top. Bacteria may be distinguished on the basis of the colony characteristics **(**Fig. 5), which may differ from species to species. Then these characteristics are compared with Barge's Manual of Systematic Bacteriology which contains the cultural characteristics for all known microorganism. This is used as the basis for separating bacteria into taxonomic groups (Tortora *et al.*, 2001).

Nutrient agar plates demonstrate well-isolated colonies and are evaluated in the following manner (Cappuccino and Sherman, 1999).

1. Size: Pinpoint, small, moderate, or large.
2. Pigmentation: Color of colony.
3. Form: The shape of the colony is described as follows:

a) Circular: Unbroken peripheral edge.
b) Irregular: Indented peripheral edge.
c) Rhizoid: Root like spreading growth.

4. Margin: The appearance of the outer edge of the colony is described as follows:

a) Entire: Sharply defined, even.
b) Lobate: Marked indentations.
c) Undulate: Wavy indentations.
d) Serrate: Tooth like appearance.
e) Filamentous: Threadlike, spreading edge.

5. Elevation: The degree to which colony growth is raised on the agar surface is described as follows:

a. Flat: Elevation not discernible.
b. Raised: Slightly elevated.
c. Convex: Dome-shaped elevation.
d. Umbonate: Raised, wth elevated convex central region.

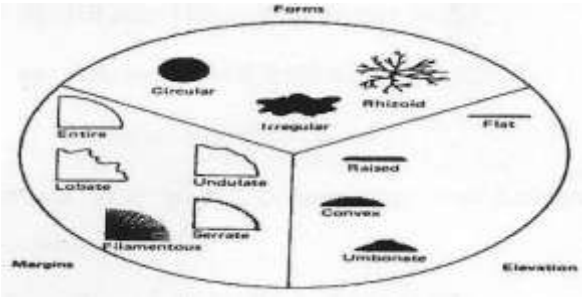

Fig. 5. Colony characteristics of bacteria (Cappuccino and Sherman, 1999).

Morphological characteristics

Morphological characteristics such as size, shape, flagellation and staining characteristics are studied by microscopic examination (Prescott *et al*., 2002).

Size

Size determinations are often indispensable in the identification of unknown bacteria **(**Fig. 6). The eubacteria vary in size from around 0.5-10 micrometers. The cyanobacteria are much larger, at times more than 10 times the size of eubacteria. For pigmented bacteria unstained water mounts are to be examined. The morphology of non pigmented bacteria are examined after staining the cells. with a suitable dye or by negative staining in which the cells remain unstained and the background is coloured with suitable dye (Prescott *et al*., 2002). The calibration procedure for the ocular micrometer requires that the graduations on both micrometers be superimposed on each other. This is accomplished by rotating the ocular lens. A determination is then made of the number of ocular divisions per known distance on the stage micrometer. Finally, the calibration factor for one ocular division is calculated as follows:

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One division on ocular micrometer (mm) = $\frac{\text{Known distance between two lines on stage}}{\text{Number of division on ocular micrometer}}$

Once the ocular micrometer is calibrated, the size of a microorganism can easily be determined, first by counting the number of spaces occupied by the organism and second by multiplying this number by the calculated calibration factor for one ocular division (Cappuccino and Sherman, 1999).

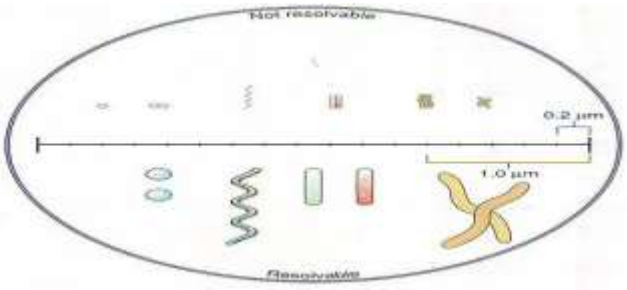

Fig. 6. Determination of microbial size (Talaro and Talaro, 1996).

Shape

Characterization of bacteria is also done on the basis of their cell structure. On this basis bacteria are categorized into three types (1) Cocci - spherical shaped cell (2) Bacilli – rod shaped bacteria, they are the most numerous in soil and (3) Spirilla- Spiral shaped cells, such type of bateria are not commonly formed in soil **(**Fig. 7) (Mishra, 2000).

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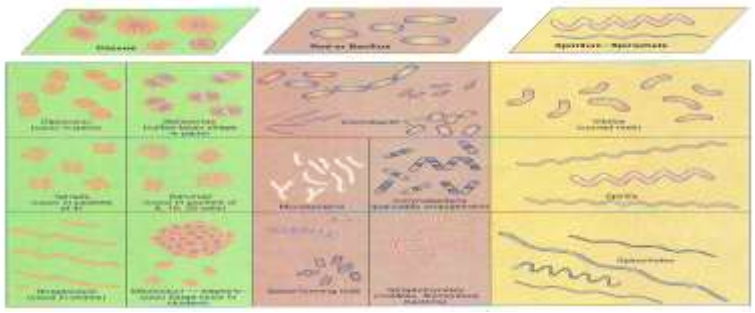

Fig. 7. Different forms of bacteria (Talaro and Talaro, 1996).

Staining

Staining characteristics give important clues in bacterial identification because it has some advantages that are i) cells are made more clearly visible after they are colored, ii) the differences between cells of different species and within the same species can be demonstrated by use of appropriate staining solution (Prescott et al., 2002). Staining techniques are two types **(**Fig. 8). Single stain is used in simple staining and two contrasting stains are used in differential staining (Cappuccino and Sherman, 1999).

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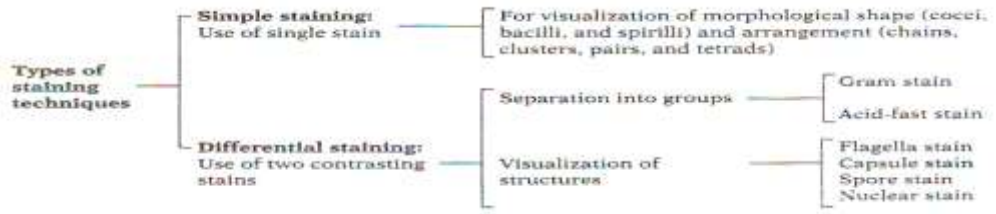

Fig. 8. Staining techniques with purposes (Cappuccino and Sherman, 1999).

Simple staining

The use of a single stain or dye to create contrast between the bacteria and the background is referred to as simple staining **(**Fig. 9). Excepting certain pigmented ones, bacteria are usually colorless and can not distinctly be seen in ordinary light microscope without a phase-contrast or dark field condenser. Thus, it is necessary to stain them with a suitable dye to study their morphology (Prescott and Harley, 2002).

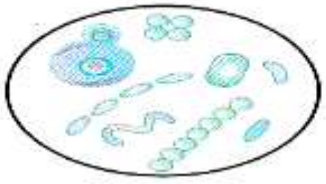

Fig. 9. Simple stain (Talaro and Talaro, 1996).

Negative staining

Bacterial cells fail to take up certain dyes like negrosinc, india ink etc. Thus when bacterial cells arc spread on a slide with such dyes, the cells appear transparent in a dark background and this type of staining of the background is called negative or background stainin **(**Fig. 10). Negative, indirect, or background staining is achieved by mixing bacteria with an acidic stain such as nigrosin, India ink, or eosin, and then spreading out the mixture on a slide to form a film. The above stains will not penetrate and stain the bacterial cells due to repulsion between the negative charge of the stains and the negatively charged bacterial wall. Instead, these stains either produce a deposit around the bacteria or produce a dark background so that the bacteria appear as unstained cells with a clear area around them (Prescott and Harley, 2002).

Fig. 10. Negative stain (Talaro and Talaro, 1996).

Gram stain

The Gram stain is the most useful and widely employed differential stain in bacteriology. It divides bacteria into two groups: Gram- negative and Gram-positive. The end result of this stain is

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that Gram -positive bacteria are deep purple in color and Gram-negative bacteria are pinkish to red in color **(**Fig. 11). The Gram stain does not always yield clear results. Gram-positive cultures may often turn Gram-negative if they get too old. Thus, it is always best to Gram stain young, vigorous cultures rather than older ones. Furthermore, some bacterial species are gram variable (Prescott and Harley, 2002).

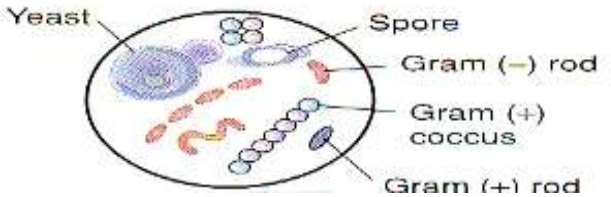

Fig. 11. Gram stain (Talaro and Talaro, 1996).

Capsule stain

Many bacteria have a slimy layer surrounding them, which is usually referred to as a capsule **(**Fig. 12). The capsule's composition, as well as its thickness, varies with individual bacterial species. Polysaccharides, polypeptides, and glycoproteins have all been found in capsules. Often, a pathogenic bacterium with a thick capsule will be more virulent than a strain with little or no capsule since the capsule protects the bacterium against the phagocytic activity of the host's phagocytic cells. However, one cannot always determine if a capsule is present by simple staining procedures, such as using negative staining and India ink An unstained area around a bacterial cell may be due to the separation of the cell from the surrounding stain upon drying (Prescott and Harley, 2002).

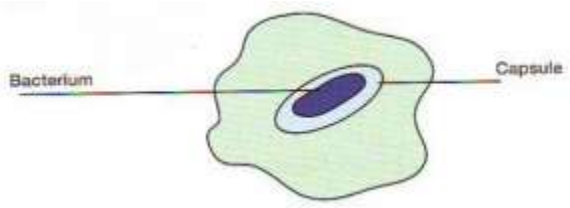

Fig. 12. Capsule with bacterium (Prescott and Harley, 2002).

Spore stain

Bacteria in genera such as *Bacillus* and *Clostridium* produce quite a resistant structure capable of surviving for long periods in an unfavorable environment and then giving rise to a new bacterial cell. This structure is called an endospore **(**Fig. 13) since it develops within the bacterial cell. Endospores are spherical to elliptical in shape and may be either smaller or larger than the parent bacterial cell. Endospore position within the cell is characteristic and may be central, sub terminal, or terminal. Endospores do not stain easily, but, once stained, they strongly resist decolorization .

This property is the basis of the Schaeffer-Fulton or Wirtz-Conklin method of staining endospores. The endospores are stained with malachite green. Heat is used to provide stain penetration. The rest of the cell is then decolorized and counterstained a

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light red with safranin (Prescott and Harley, 2002).

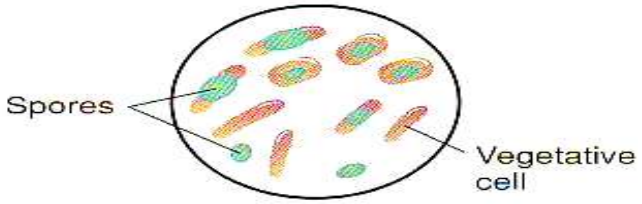

Fig. 13. Spore stain (Talaro and Talaro, 1996).

Acid fast stain

A few species of bacteria in the genera *Mycobacterium* and *Nocardia* and the parasite *Cryptosporidium* do not readily stain with simple stains. However, these microorganisms can be stained by heating them with carbolfuchsin. The heat drives the stain into the cells. Once the microorganisms have taken up the carbolfuchsin, they are not easily decolorized by acid-alcohol, and hence are termed acid-fast **(**Fig. 14). This acid-fastness is due to the high lipid content in the cell wall of these microorganisms (Prescott and Harley, 2002).

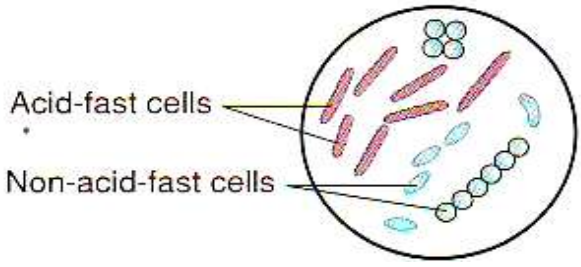

Fig. 14. Acid fast stain (Talaro and Talaro, 1996).

Biochemical characteristics

Identification of a microbial isolate usually follows from a sequence of morphological, biochemical, immunological, and genetic techniques. Delineation of the biochemical activities of a microbial isolate is the most important and convenient way to narrow the search path towards the identity of an unknown strain. Biochemical reactions, for the purpose of identification, often utilize a limited combination of metabolic and enzymatic activities prominent and pertinent to a specific group of microorganisms that share these common activities. Variations in biochemical activities exhibited by members of the same group of organisms are then utilized to differentiate between these individuals and ultimately lead to identification. Yet, each living organism is unique in its biochemical activities. The cells perform a number of enzyme-mediated reactions which are together termed metabolism. Presence or absences of certain enzymes, intermediary metabolites or end products often give valuable information for the identification of an organism. A 21 number of biochemical tests are available which help to identify the biochemical activity of bacteria and through which bacteria can be classified. Among the most commonly utilized microbial biochemical activities are

1) Fermentation or oxidation of certain carbohydrates
2) Hydrolysis of starch and cellulose

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3) Gelatin liquefaction (or hydrolysis)

4) Production of indole, hydrogen sulphide, acetyl methyl carbinol, etc. in media
5) Hydrolysis of coagulated serum and casein
6) Reduction of nitrate, sulphate, methylene blue or litmus in media
7) Production of specific enzymes (which can be assayed) such as catalase, indophenol oxidase, amino acid deaminase and decarboxylase, urease, celullase, alpha and beta amylases, phosphatase, hyaluronidase, lecithinase, etc (Sullia and Shantharam, 1998).

Since the 1970s, routine sugar rack biochemical tests performed in test tubes headed towards miniaturization. In addition to saving materials, miniaturization enabled the expansion of the number of tests that could be included in a single test panel for a specific group of microorganisms. Furthermore, expansion of the number of tests in biochemical identification schemes led eventually to the development of extensive databases associating biochemical parameters with the identity of microbial species. Many commercial miniaturized biochemical test panels are now available in the market; they cover a significant number of clinically-important groups of bacteria, as well as food- and water-associated microorganisms. Among these test panels, and maybe one of the earliest, is the Analytical Profile Index (API) panel. The API 20E System is a standardized, miniaturized version of convectional biochemical procedures used in the identification of *Enerobacteriaceae* and other gram-negative bacteria. A total of 127 taxa can be identified with this system. It is a ready-to-use, micro tube system that performs 23 standard biochemical tests on pure bacterial cultures from appropriate primary isolation media. Besides morphological, colonial and biochemical characteristics, some other parameters also taken into consideration for the rapid identification of bacteria are the following cultural, physiological, pathogenicity and ecological characteristics (Pankhurst, 1978).

Conclusion

The diversity of soil bacteria reflects the complexity of their ecological roles. Traditional isolation and staining techniques continue to be invaluable tools for understanding bacterial taxonomy and physiology. However, limitations exist—many soil bacteria remain unculturable using standard laboratory media. Thus, combining classical microbiological methods with molecular approaches (e.g., 16S RRNA sequencing) enhances the accuracy of bacterial identification. In developing nations, including Bangladesh, comprehensive bacterial profiling can contribute to improved soil management and sustainable agricultural practices. The isolation and characterization of indigenous bacterial strains may also reveal species with potential for biofertilization, biocontrol, or bioremediation applications. Soil bacteria are indispensable to ecosystem health, influencing nutrient cycling, decomposition, and soil fertility. Isolation and characterization techniques—ranging from colony observation to staining and biochemical assays—provide foundational insights into their taxonomy and function. Continued research integrating classical microbiology with modern molecular tools is essential for a fuller understanding of bacterial diversity and its practical applications in agriculture and environmental management.

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