Subject : Biochemistry

Semester : IV

Name Of the Teacher : Dr. Ruma Das

Name of the Topic : Core Course 10

Basic Microbiology

Microorganisms in Biological world

Eubacteria : A general feature

- Eubacteria or true bacteria are single celled prokaryotic microorganism and they are lacking a defined membrane bound nucleus.
- They have a cell wall made of peptidoglycan and this provide the necessary strength needed to maintain its shape and size during changes in environment.
- They do have a plasma membrane similar to eukaryotes.
- Some bacteria may have a flagella or cilia for movement
- Cytoplasm is enclosed within the plasma membrane
- They donot have membrane bound mitochondria or chloroplast.
- Bacterial DNA floats freely into cytoplasm.
- Bacteria can reproduce asexually through binary fission.
- Sometimes they can produce endospore which can tolerate extreme hostile environment and hard to kill those endospores.

Ex. of eubacteria — E.coli, S. pneumoniae.

Archaebacteria : A general feature

- Archaebacteria are primitive single celled microorganism living in extreme environments.

- They are considered to be evolved just after the first life on earth and hence are called ancient baeleria.
- They are found in hot springs, salt lakes, oceans, soils as well as in human skin, oral cavity and colon as well.
- Three types of archaebaeleria are found methanogens, halophiles and thermophiles.

Methanogens are found in oxygen free environment and produces methane gas. Halophiles live in water with high conc. of salts.
Thermophiles live in hot environment

Major differences between Eubaeleria & Archaebacli

Characteristic	Archae	Eubac
• Cell Wall Peptidoglycan containing muramic acid and D-amino acids is present	−	+
• Membrane lipid FA bound to glycerol by <u>ester</u> linkages	−	+
FA bound to glycerol by ether linkages	+	−
• <u>Introns</u>	+	−
• First amino acid to initiate new polypeptide chain	Met	F Met
• Habitat	Extreme condition	Everywhere on earth

Bacterial Morphology

The bacteria are an extremely diverse collection of prokaryotic microorganisms, exhibiting greatly different morphologies and physiologies.
Depending on the shape of bacteria they can be classified as

i) coccoid - spherical shape
* ii) rods - cylindrical shape
* iii) spirilla - spirals shape
iv) pleomorphic - irregular shape

* rods - may be straight rod - termed bacilli
rods - may be helically curved - termed spirilla.

Bacterial cells are usually arranged in a manner characteristics of their particular species. Allhough all cells are not arranged in same manner but predominant- arrangement is an important feature.

Cocci appear in several characteristics arrangement. They may be a) diplococci - cells are mostly paired. b) streptococci - cells are attached to form a chain.
c) Tetracocci - cells divide in two planes

and characteristically form groups of four cell.

d) Staphylococci - cells divide in three planes, producing bunches of cocci (irregular pattern)

e) Sarcinae - cells divide in three planes in a regular pattern, producing a cuboidal shape of arrangement.

Bacilli may occur singly or pairs called diplobacilli. Sometimes they form chains and termed as streptobacilli. Some bacillus sp. cells are arranged side by side like a matchstick, are known as palisade arrangement.

Curved bacteria may take the shape of 'comma' are termed as vibriods or they may be helical.

In addition to the common bacterial shapes, so many different shapes of bacteria are also found in nature.

Subcellular structure of bacteria

Bacterial cells reveals various components e.g cell wall, cytoplasmic membrane, cytoplasm, Nuclear material, ribosome, spores and cysts.

Cell walls are naturally common to almost all bacteria. Bacterial flagella are hairlike helical appendages that protrude through the cell wall, are responsible for swimming motility. Certain helical bacteria exhibit motility though they lack external flagella. For them, flagella like structures located within the cell, just beneath the outer cell envelop and are termed as periplasmic flagella or axial fibril or endoflagella. Pili are hollow, nonhelical filamentous appendages attached to cell wall bacteria. Some pili help attachment of the bacteria to host cell whereas some serve as port of entry of genetic material during bacterial mating (F pili) Sometimes bacterial cells are surrounded by a viscous substance known as capsule and capsules serve several functions e.g they may promote attachment of bacteria to surfaces or inhibit the engulfment of pathogenic bacteria by white blood cell etc.

Cytoplasmic membrane - It is a layer of phospholipid and proteins, underneith the cell wall and encloses the interior of the bacterium. Membrane are dynamic in nature and serve as a barrier to different molecules.

Nucleoid - The nucleoid is a region of cytoplasm where chromosomal DNA is located. The nucleus is not membrane bound but simply an area of the cytoplasm where the strands of DNA are found. Small circular plasmid DNA are also found in the cytoplasm.

Ribosomes - Bacteria contain ribosome and distributed to all over cytoplasm. Ribosome function in protein synthesis of the bacteria. Bacterial ribosome is different in composition than eukaryotic ribosome.

Spores and Cysts - Certain species of bacteria produce spores, either within the cell (endospore) or external to the cell (exospores). The spore is a metabolically dormant form, which under appropriate conditions, undergo germination and form vegetative cells.

Cysts are dormant thick walled, dessication resistant forms that develop by differentiation of vegetative cell. Though they resemble endospores but do not have the high heat

Microbial Staining

A large number of coloured organic compounds (dyes) are available for staining microorganisms. These compounds may be <u>acidic</u>, <u>basic</u> or neutral. An acid dye (or anionic) is one in which the charge on the dye ion is negative, a basic (or cationic) dye is one in which the charge carried by the dye is positive. A neutral dye is a complex salt of a dye acid with a dye base, e.g. eosinate of methylene blue. Acid dyes generally stain basic cell components and basic dyes generally stain acidic cell components.

Acidic dye (Nigrosin, Eosin, Acid Fuchsin, India ink)

Basic dye (crystal violet, Methylene blue, safranin, basic fuchsin)

<u>Staining Theory</u> : The process of staining may involve ion-exchange reactions between the stain and the active sites at the surface of or within the cell. For ex. the coloured ions of the dye may replace other ions on cellular components. The protein or Nucleic acid part of bacterial cell may form a salt formation and can be represented as

$$[\text{Bacterial cell}]^{-}\ Na^{+}$$

In a basic dye like methylene blue, the coloured ion is positively charged (a cation) & represented by MB^{+} and it forms a salt with Chloride & becomes $MB^{+}Cl^{-}$

Thus by ion exchange principle, following reaction occur,

$$(\text{Bacterial cell}^-)\,Na^+ + (MB^+)\,Cl^- \rightarrow (\text{Bacterial cell}^-)\,MB^+ + Na^+\,Cl^-$$

Here, MB^+ cation replaces the Na^+ cation in the cell.

Commonly used stains are methylene blue (blue), crystal violet (purple) and safranin (red)

Staining can be two types — i) Simple staining ii) differential staining

In simple staining procedure, a single stain is used and all cells and subcellular structures generally stain the same colour. Simple staining is further divided into positive and negative staining.

Positive staining - Here, the stain, is basic and has a positively charged chromophore (coloured portion of the stain molecule), that is attracted to the negatively charged outer surface of the microbial cell. A stain e.g. methylene blue has a blue chromophore, resulting in a positive blue staining of the microorganism.

Negative staining - Here, the stain is acidic, has a negatively charged chromophore, that is repelled by the negatively charged microorganisms so that it

colours the background, resulting in the apparent-negative or indirect staining of the microbial cell. Nigrosine and India ink are frequently used for negative staining of microbial cells.

Differential Staining

In this technique, multiple staining reactions are employed. Differential stains take advantage of the fact that specific types of microorganism or the particular structures of a microorganism exhibit different staining reaction that can be readily distinguished by their different colors. Example of differential staining.

i) Gram staining - This staining procedure begins with a primary stain using crystal violet, which stains all bacterial cells blue-purple, followed by application of Gram's iodine - iodine is a mordant that fixes the primary stain in bacterial cell. The the bacteria is decolourized with acetone-alcohol, which remove primary stain and the counterstain is achieved with the application of red safranin stain. Gram staining procedure results in blue-purple colouration of bacteria, whereas gram negative gives red pink colour.

The structure and composition of gram +ve & -ve bacteria are different. Lipid content of -ve bacteria is more than gram +ve. During washing with alcohol, lipid is extracted from cell wall and resulting in increased permeability of the cell wall of gram -ve bacteria. Thus CV-I complex can be extracted and organism decoloured and subsequently take safranin stain.

On the other hand, being lower lipid content in gram +ve bacterial cell wall, it becom dehydrated during alcohol treatment. Then the pore size decreases, permeability decreases, CV-I complex cannot be extracted and hence the gram +ve bacterial remain violet.

Furthermore, Gram +ve bacteria have a thick peptidoglycan layer in its cell wall compared to gram -ve one. Thus a bacterial cell with a gram +ve cell wall having thick peptidoglycan layer traps the stain but the gram -ve wall with its thin peptidoglycan layer does not.

i) Acid-fast staining

This is another differential staining procedure. In this procedure, cells are initially stained with carbol fuchsin and then decolonization is performed with acid alcohol.

Acid fast bacteria retain the red colour of the carbol fuchsin and are not decolourized. Non-acid-fast bacteria are decolourized and when counterstained with methylene blue. they appear as blue stain containing bacteria This procedure is specially useful in identifying the causative agent of tuberculosis - Mycobacterium tuberculosis, which is acid fast. Acid fast mycobacteria contain mycolic acid in their outer membrane, making the cell waxy and resistant to staining aqueous based stain e.g gram stain. The primary stain, carbolfuchsin is applied to the cells, and heat and phenol are used to allow the stain to penetrate into the waxy surface of acid fast microorganisms. The excess stain is then removed by acid alcohol treatment. A secondary stain, methylene blue, is then applied to the cells.

Endospore Staining

This is also a differential staining. Endospores are not easily stained and remains colourless while the rest of the cell is stained. Endospores, can be stained using malachite green and steam to drive the stain into the endospore. Once stained the endospore

resists decolourization. In a typical endospore-staining procedure, malachite green is used as a primary stain and water as decolouring agent. Water can wash the primary stain out of the vegetative cells but not the endospores. The pink safranin is then used to stain the vegetative cells. Thus at the end, endospore is stained green and rest of the bacterial cell is stained pink, permitting differentiation of endospores from vegetative cell. Malachite green is water soluble and has a low affinity for cellular material, so vegetative cells may be decolonized with water.

Auxochrome: An auxochrome is a group of atoms attached to a chromophore which modifies the ability of the particular chromophore to absorb light.

Chromophore: The term chromophore is used to denote a functional group of which gives a colour to a compound.
The term can be used in a broader sense i.e any group that exhibits absorption of electromagnetic radiation in a visible or ultra-visible region.

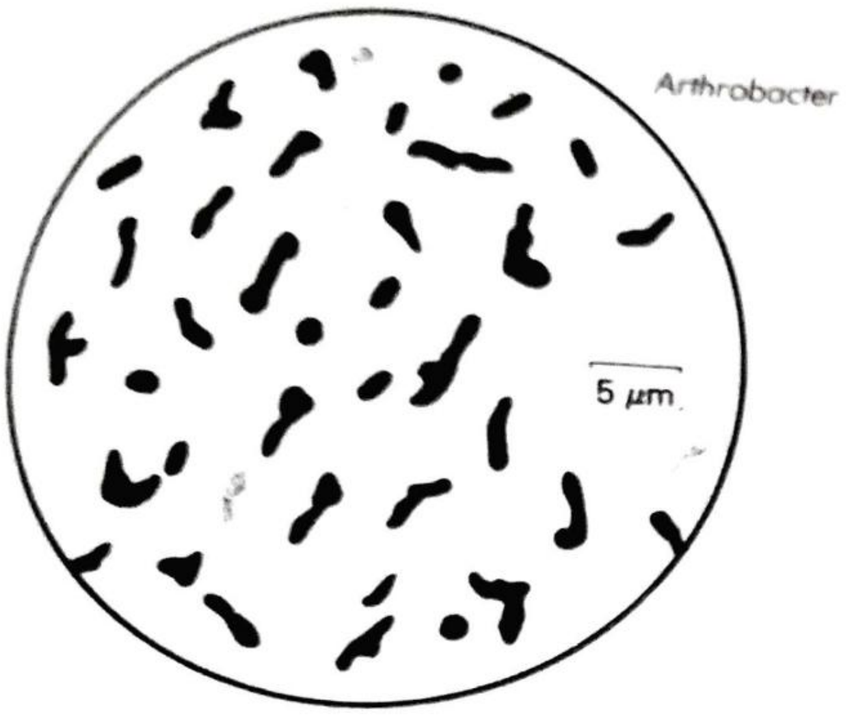

Figure 5-2. Drawing of pleomorphic cells of the genus *Arthrobacter*. *(Erwin F. Lessel, illustrator.)*

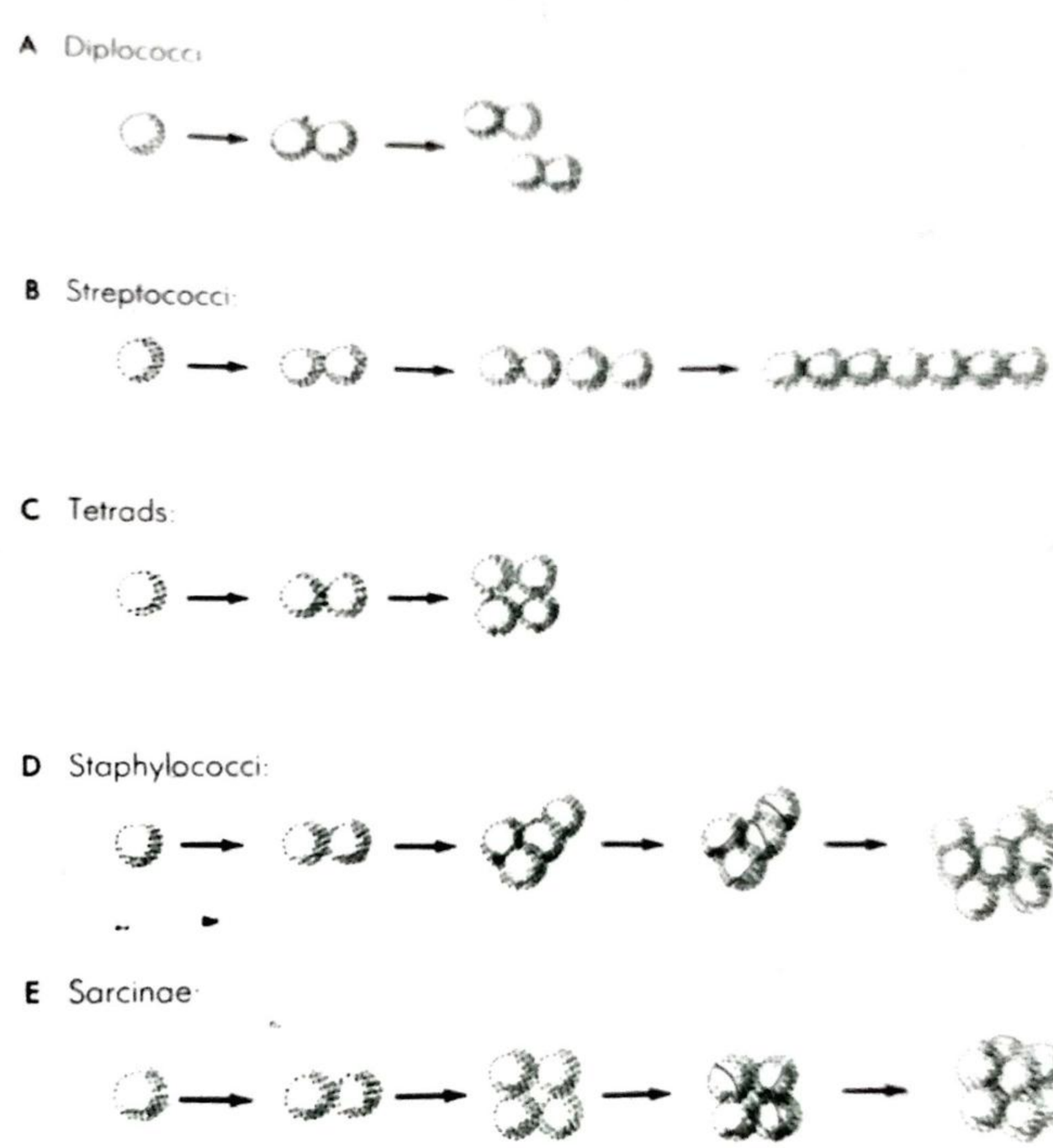

Figure 5-3. Characteristic arrangements of cocci, with schematic illustrations of patterns of multiplication. (A) Diplococci: cells divide in one plane and remain attached predominantly in pairs. (B) Streptococci: cells divide in one plane and remain attached to form chains. (C) Tetracocci: cells divide in two planes and characteristically form groups of four cells. (D) Staphylococci: cells divide in three planes, in an irregular pattern, producing "bunches" of cocci. (E) Sarcinae: cells divide in three planes, in a regular pattern, producing a cuboidal arrangement of cells.

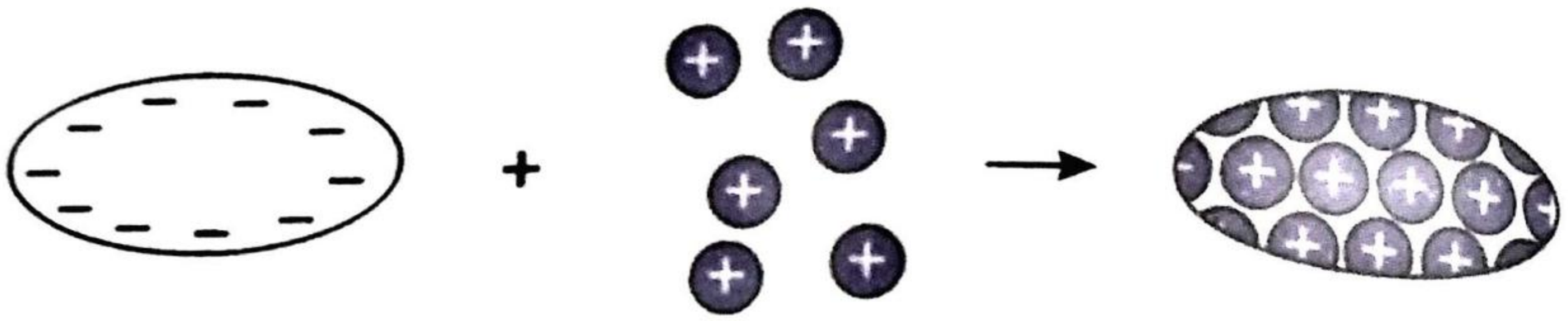

Fig. 2-8 Simple Staining Procedure—Positive Staining. **A,** In a simple staining procedure, microorganisms are affixed to a glass slide and stained with an appropriate dye (colored chromophore). This increases the contrast between the cells and the background so they can be seen easily using a light microscope. Because the outer layer of a cell is negatively charged, a positively charged stain chromophore is attracted to the cell; this is the basis of positive staining procedures. **B,** Micrograph of the bacterium *Bacillus cereus* after simple positive staining with carbol fuchsin. (1,300×.) The cells appear red in contrast to the clear background.

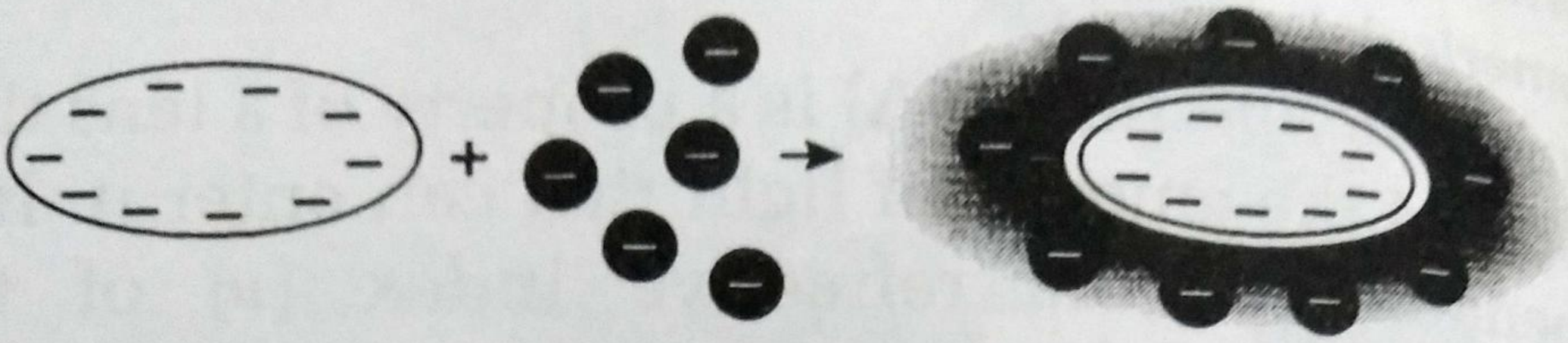

Fig. 2-9 Simple Staining Procedure—Negative Staining. **A,** Because the outer layer of a cell is negatively charged, a negatively charged stain chromophore is repelled by the cell; this is the basis of negative staining procedures. **B,** Micrograph of the bacterium *Escherichia coli* after simple negative staining with India ink. The cells appear clear against a dark background.

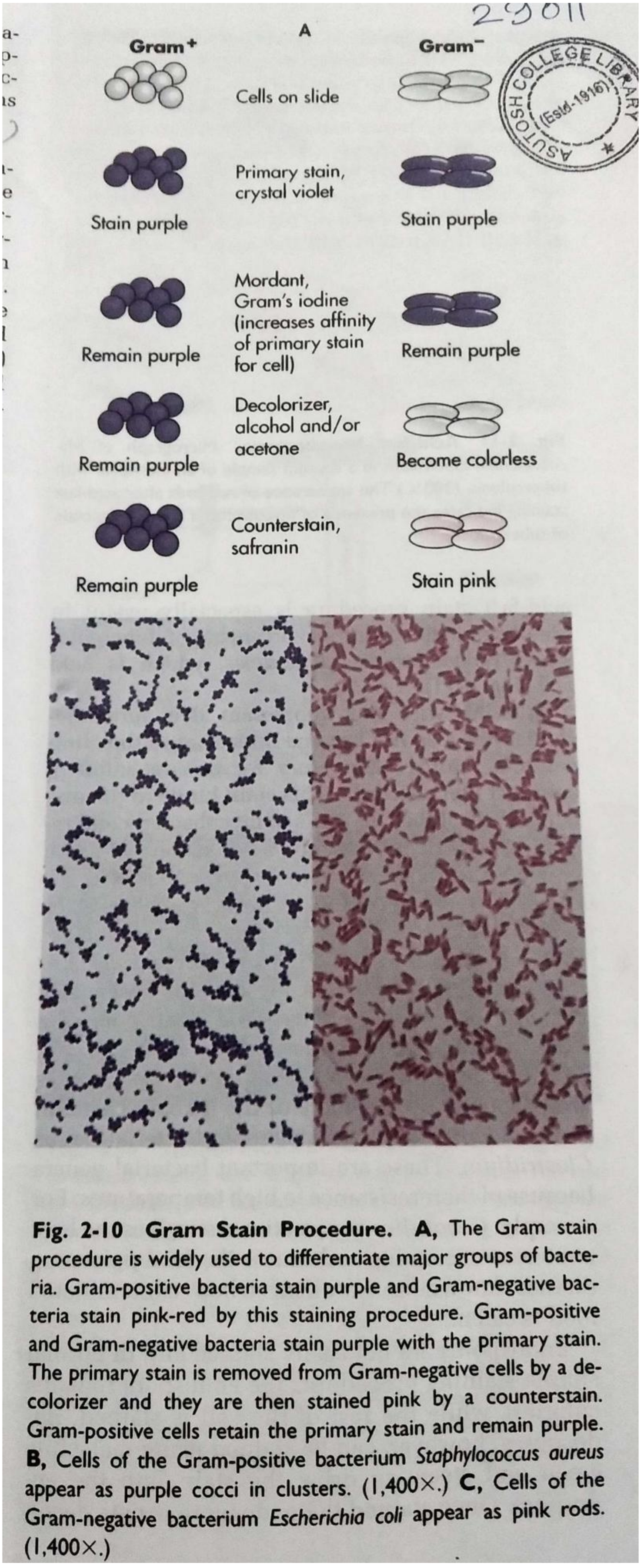

Fig. 2-10 Gram Stain Procedure. **A,** The Gram stain procedure is widely used to differentiate major groups of bacteria. Gram-positive bacteria stain purple and Gram-negative bacteria stain pink-red by this staining procedure. Gram-positive and Gram-negative bacteria stain purple with the primary stain. The primary stain is removed from Gram-negative cells by a decolorizer and they are then stained pink by a counterstain. Gram-positive cells retain the primary stain and remain purple. **B,** Cells of the Gram-positive bacterium *Staphylococcus aureus* appear as purple cocci in clusters. (1,400×.) **C,** Cells of the Gram-negative bacterium *Escherichia coli* appear as pink rods. (1,400×.)

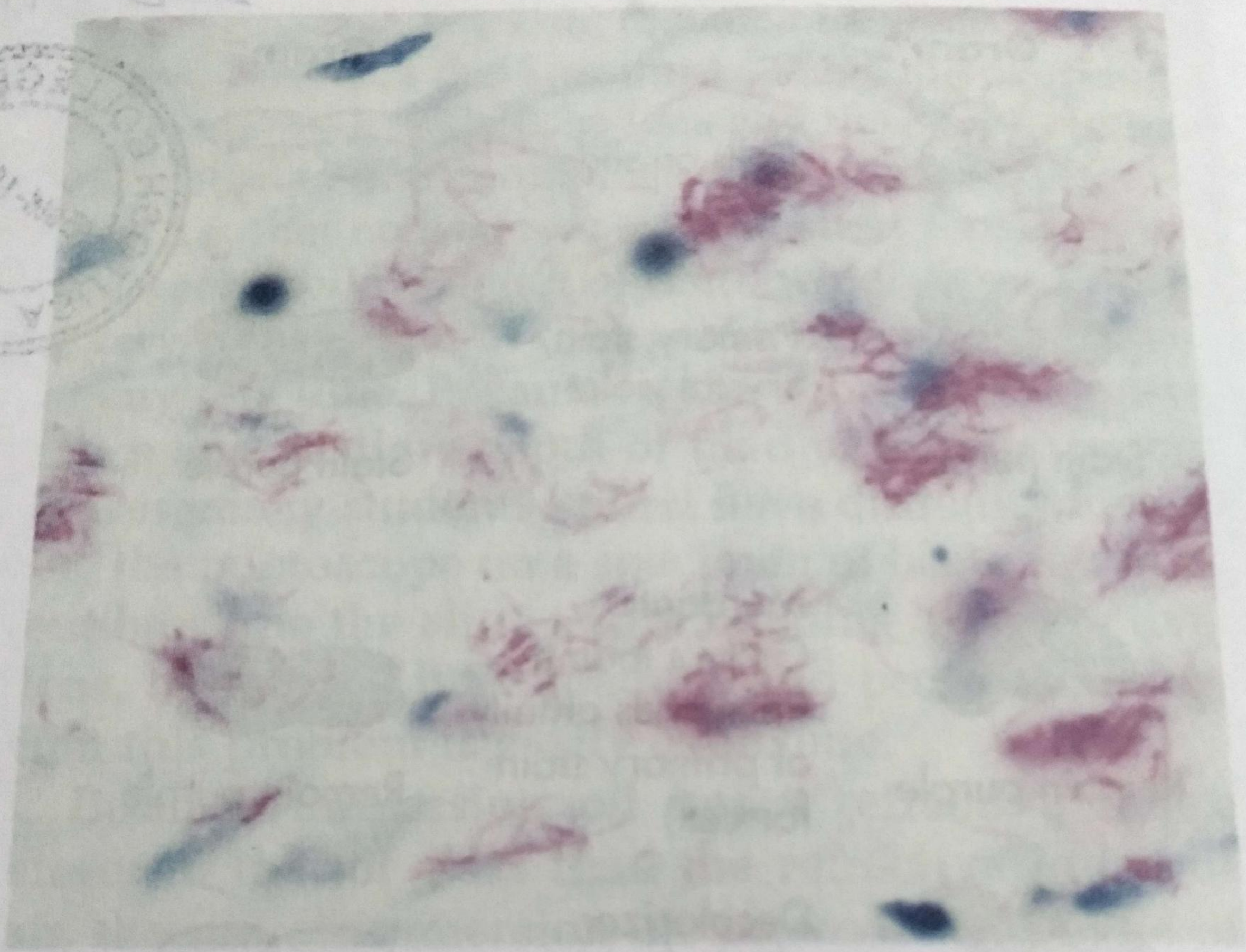

Fig. 2-11 Acid-fast Mycobacteria. Micrograph of *Mycobacterium tuberculosis* in a sputum sample of an individual with tuberculosis. (300×.) The appearance of red rods after acid-fast staining indicates the presence of mycobacteria and is diagnostic of tuberculosis.

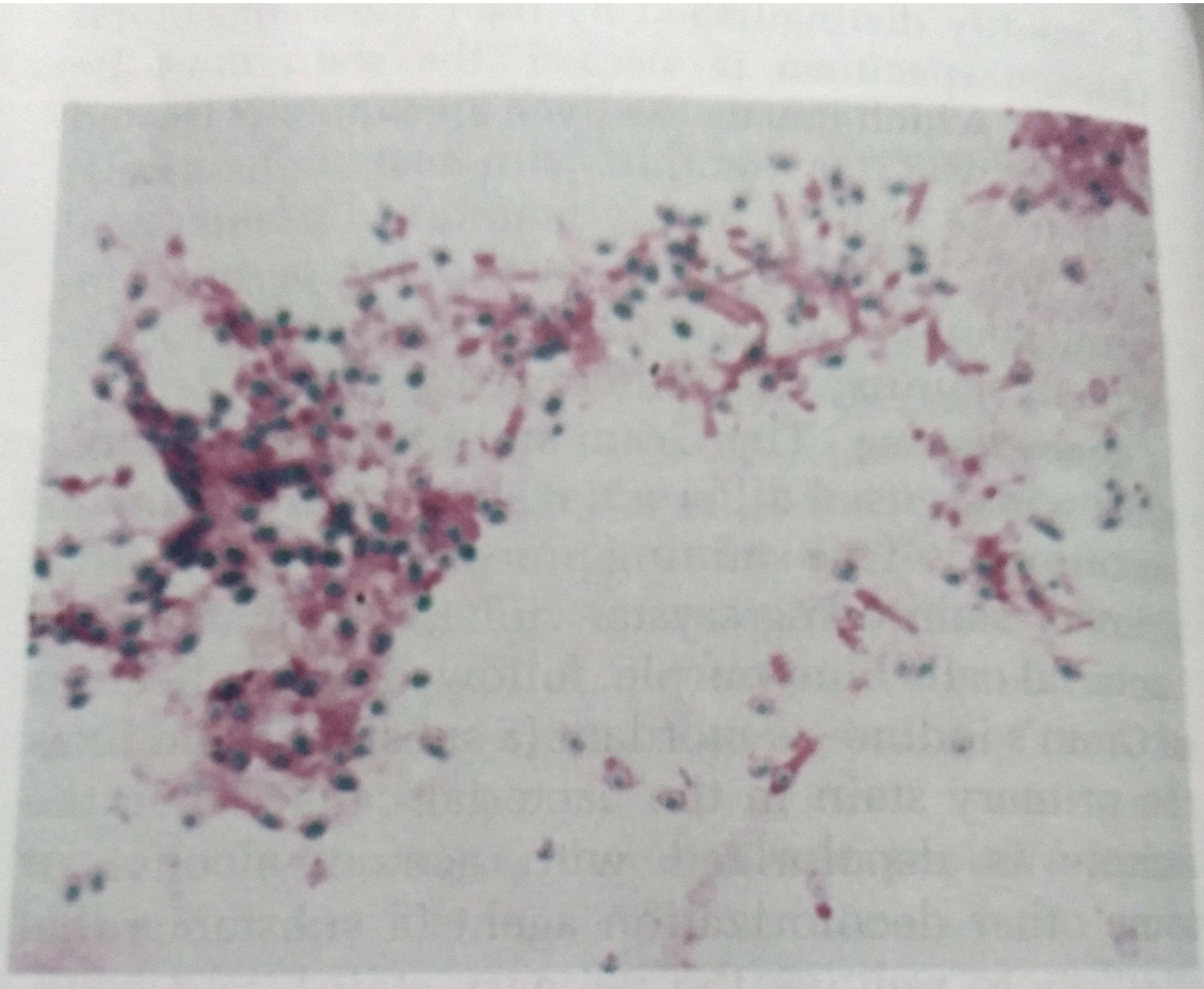

Fig. 2-12 Endospores of Clostridia. Micrograph of *Clostridium tetani* after endospore staining. (1,400×.) The spores appear green and the bacterial cells are stained red.