Continue …….
Microbiology (2) (205 M) Bacteria Chemical composition of bacterial cell

By

Mohamed Ismail Abou-Dobara

Professor of Bacteria, Faculty of Science, Botany and Microbiology Department, Damietta University

- At the end of this lecture the student must able to:
- 1- describe the nature of cytoplasmic inclusions.
- 2- describe the sporulation process of bacterial endospore.
- 3- list some endospore producing bacteria.
- 4- describe and the importance of the endospore.
- 5- compare between the physiological properties of the vegetative cell and endospore.

- Lecture 6
- The contents
- 1-The nature of the cytoplasmic inclusions of the bacterial cell.
- 2-Bacterial endospore.

CYTOPLASMIC INCLUSIONS

- **Various granules or inclusions are present in the cells of an aging bacterial culture.**
- **Inclusions are nonliving bodies in the cytoplasm.**
- **Many seem to be reserve food materials because they accumulate during conditions of good nutrient supply and decrease during starvation.**

- **The nature of the inclusions varies with the organism.**
- **Volutin granules, sometimes called metachromatic granules, appear in various bacterial species and also in many fungi, algae, and protozoa; they stain intensely with basic dyes and contain polymerized phosphoric acid.**

- Polysaccharides may accumulate as glycogen or as a kind of starch. Lipid globules appear in various bacteria, particularly gram-positive organisms. Sulfur and iron are also found in certain species.

Endospores

- **Endospores are highly resistant bodies produced within the cells of certain bacteria.**
- **One bacterial cell normally produces only a single endospore. Sporulation is therefore not considered to be a method of multiplication of bacteria as it is of yeasts and molds.**

- **Endospore are found in all species of the family Bacillaceae,**
- **The endospore producing bacteria include:**
- **1-*Bacillus* (aerobic spore-forming rods);**
- **2-*Clostridium* (anaerobic spore-forming rods);**
- **3-*Sporosarcina* (spore-forming cocci);**
- **4-*Desulfotomaculum* (anaerobic, sulfate actively reduced to sulfide rods); and**
- **5-*Thermoactinomyces* (filamentous bacteria, previously included in actinomycetes).**

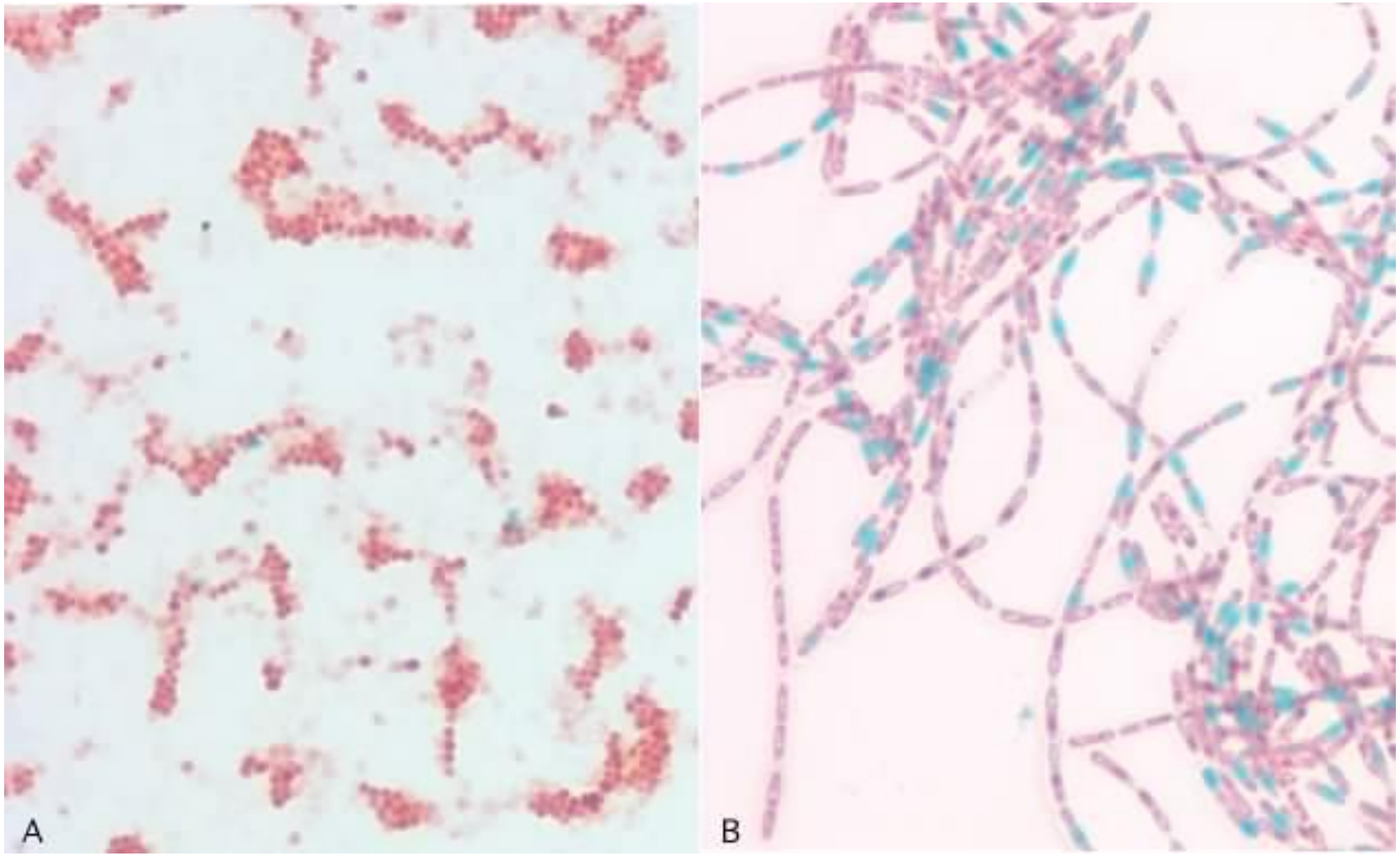
Physical and Physiologic Characteristics **of endospore**

- **Endospores are spherical and may be situated anywhere within the parent cell or sporangium.**
- **Their diameter may be less than, equal to, or greater than that of the rest of the sporangium.**

- **A cell with a greatly enlarged central endospore resembles a spindle and is called a clostridium.**
- **A plectridium is a sporangium containing an enlarged terminal endospore.**
- **The sizes of spores differ from one species to another; this property is of some use as a criterion for classification.**

- **Unstained bacterial endospore are highly refractile when observed with the microscope.**
- **Ordinary simple staining methods color only the outer layer or spore coat.**
- **The inside of a spore can be stained if heat is applied.**

- **Apparently this treatment increases the permeability of the spore envelopes and permits strong dyes such as malachite green as in the following Figigure or carbol fuchsin to penetrate and stain the cytoplasm intensely.**
- **Stained endospore resist decolorization and are easily distinguished from vegetative cells or from other portions of sporangia.**


A
B

- Fig ( ) A photomicrograph of an enodspore stain. Spores present in the picture stain green, while the vegetative cells stain red. A) *Staphylococcus epdiermidis* which does not form endospores. B) The endospore-forming rod, *Bacillus cereus*.

Sporulation

- **In a vegetatively multiplying cell, the DNA replicates and divides by processes that will be described later, and each half of the DNA comprises the nuclear material of a new vegetative cell.**
- **This cycle of DNA and cellular replication continues as long as growth conditions remain favorable.**

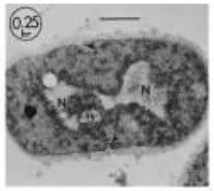
- **When conditions change and favor sporulation, half of the DNA (i.e., a complete genome of the longitudinally distributed nuclear body of the stage I cell)**
- **becomes segregated into a compartment at one end of the cell, where it is separated from the remaining nuclear and cytoplasmic material by a spore septum (stage II).**

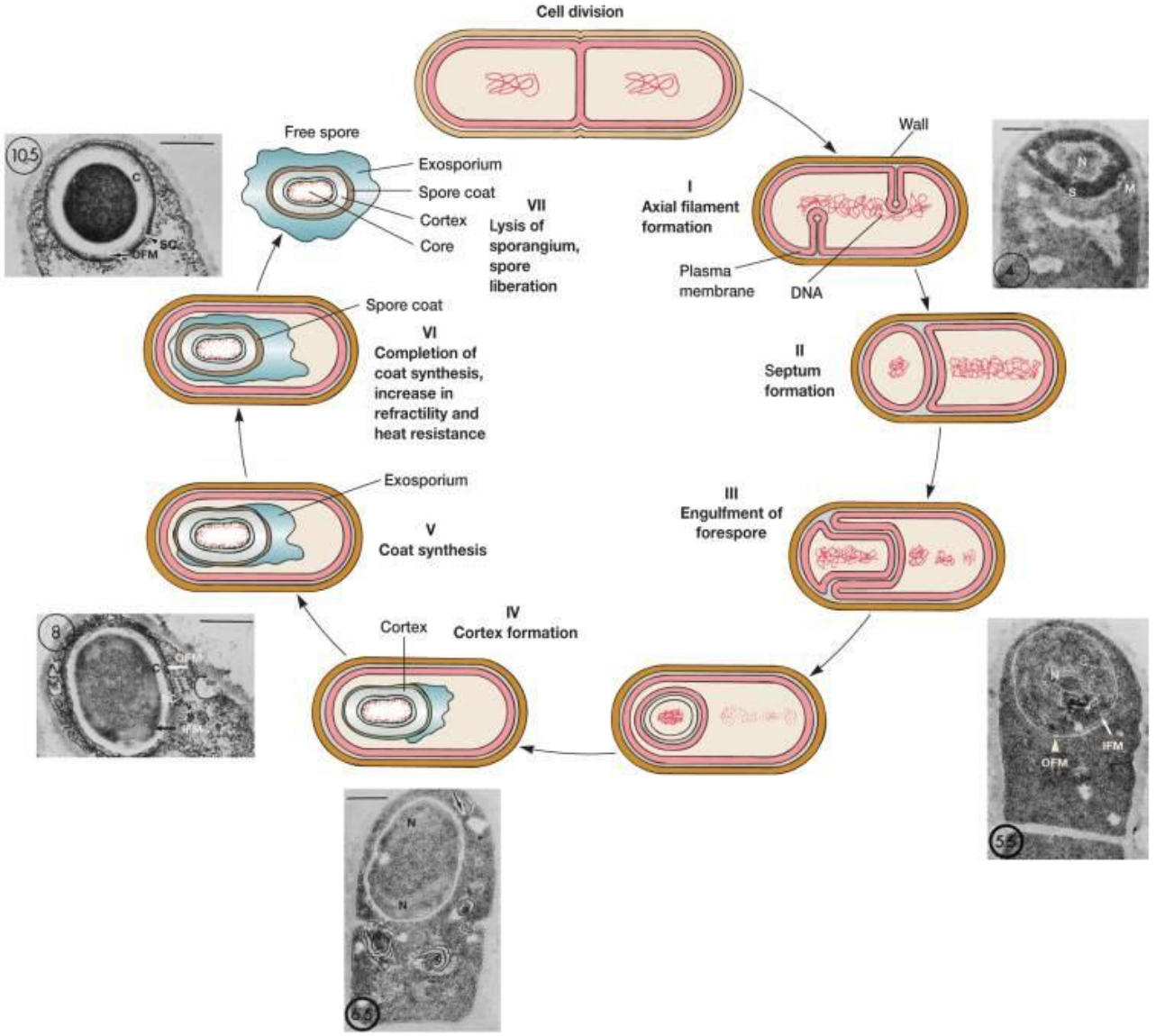
- **This compartment develops into a primordial spore or “forespore” (stage III).**
- **From this point the two portions of the cell constitute different metabolic systems.**
- **Although each contains half the DNA of the original cell, replication of DNA in the remainder of the sporangium of certain species may continue to replicate until lysis occurs, and vegetative components continue to be produced.**

- **Biosynthetic activity in the forespore yields only spore-specific components, which differ from vegetative components.**
- **New envelopes then from around the forespore: the cortex, immediately outside the spore wall (stage IV), and one or two spores coats outside the cortex (stage V).**

- **The nucleus becomes somewhat less dense as it is distributed throughout the spore cytoplasm.**
- **The structure of the cortex changes as calcium dipicolinate accumulates, the outer portion of the cortex becomes less dense, and the thermo resistance of the spore increases (stage VI).**

- **The stages in sporulation are illustrated in the following figure (**Endospore formation in *Bacillus megaterium)***:**





- **The fine structure of endospore in the following Figure differs somewhat from one species to another in general however, there is a central core consisting of the spore cytoplasm and nuclear material.**
- **This is surrounded by a delicate membrane and the spore wall. In many species the spore wall will eventually transform into the cell wall of the future bacillus.**

- **In many species the spore wall will eventually transform into the cell wall of the future bacillus. Around the wall is a second layer, thicker and of relatively low density, the cortex.**
- **This is the location of dipicolinic acid, mucopeplide polymers, and calcium, all of which are significant in spore resistance.**
- **The cortex, in turn is enclosed in one or two (depending on the species) spore coats.**

- **Spore may be smooth grooved, or raised into ridges, sometimes in geometric (e.g., hexagonal) patterns.**
- **Lastly, the whole is enclosed in an exosporium, which may be somewhat wrinkled and enclose some cytoplasm material derived from the sporangium.**

- **Endospores are the most resistant of all living bodies to heat desiccation, and toxic chemicals, but there is great variation among species.**
- **Some endospores are killed within a few minutes at 80^{o} to 90^{o} C, whereas those of other species survive prolonged boiling. Spores of one bacillus resist 100^{o} C, for over 20 hours.**

- **The remarkable resistance of endospores implies that their chemical composition or physical structure must differ radically from that of the parent cells.**
- **Chemical analysis reveals that endospores contain DNA and RNA, proteins, lipids, carbohydrates, various enzymes, and minerals. Their water content is approximately 25 per cent less than that of vegetative cells.**

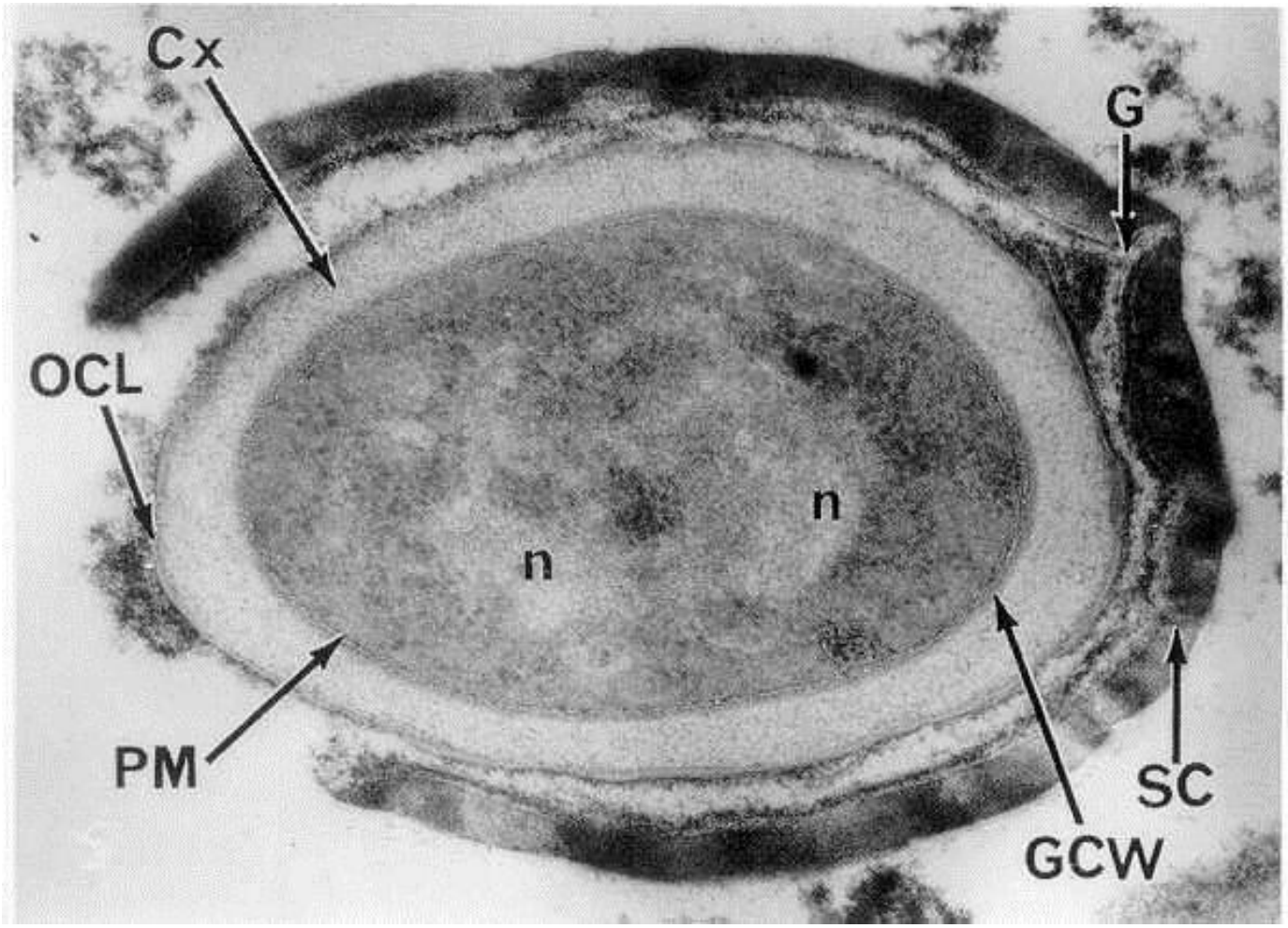
- **It has long been suggested that the ratio of “bound” to “free” water in spores is greater than in vegetative cells.**
- **In addition, spores contain more calcium, some of the proteins are the same as those in vegetative cells, but numerous proteins to endospores have been found.**

- **Formation of an endospore involves new synthesis of proteins, including enzymes, as well as incorporation of vegetative cell constituents.**
- **Some spore enzymes differ qualitatively from their vegetative cell counterparts.**
- **For example, catalase from vegetative cells is soluble and heat-sensitive, whereas that from spores is attached to particles and is resistant to heat.**

- **One of the most striking features of endospore is a compound, dipicolinic acid, that is present in the cortex of spores and absent from all vegetative cells. It makes up 5 to 15 per cent of the dry weight of the spore.**
- **Dipicolinic acid, peptides, and other substances are released from germinating spores, coincident with loss of resistance.**

- **It therefore appears that dipicolinic acid is partly responsible for spore resistance.**
- **The metabolic activity of endospore is very low. They contain several active enzymes, but many others are present in a dormant or inactive state.**
- **There is evidence that some of the enzymes and other normally thermolabile substances within spores are bound in the form of chemical complexes with dipicolinic acid and perhaps also peptide and calcium. These complexes are highly resistant.**

- **Other factors contribute to the resistance and low metabolic activity of spores.**
- **The impermeability of the spore coat undoubtedly prevents the entrance of lethal chemicals.**
- **The dehydrated endospore cytoplasm is unfavorable for any kind of chemical activity.**


Cx
G
OCL
n
n
PM
SC
GCW

- Fig ( ) Electron micrograph of a thin section of a *Bacillus megaterium* spore showing the thick spore coat (SC), germinal groove (G) in the spore coat, outer cortex layer (OCL) and cortex (Cx) germinal cell wall layer (GCW), underlying spore protoplast membrane (PM), and regions where the nucleoid (n) is visible.

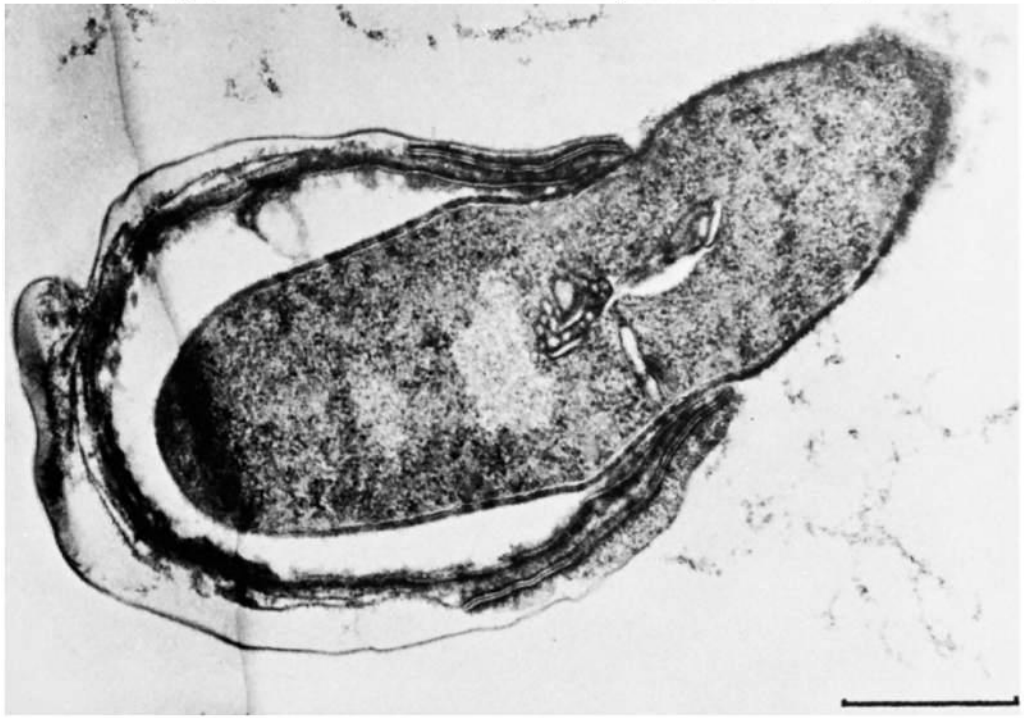
Transformation of Endospore into Vegetative cells

- **The transformation of endospores into vegetative cells as in the following Figure occurs within a very few hours after transfer to a favorable environment.**
- **This is true whether the spores have only recently been formed or have been dormant for a long time.**

- **Spores can survive for many years; bacteriologist is so young that no one knows just how long.**
- **Favorable conditions include the presence of water and nutrients, suitable temperature and oxygen tension, and the presence of certain “trigger” substances and conditions.**
- **Three processes occur in sequence during the transformation of the spore into the vegetative cell:**

- a) Activation conditions the spore to germinate in a suitable environment;
- b) Germination is a process in which the typical characteristics of a dormant spore are lost; and
- c) Outgrowth, characterized by the formation of new proteins and structures, converts the spore into a vegetative cell.

- The following figure show ndospore germination of *Clostridium pectinovorum*.



Questions

- 1- Choose the correct answer:
- (i) **Which of the following statements about endospores is false?**
- **(i) they are formed in response to adverse environmental conditions.**
- **(ii) they provide a means of asexual reproduction for bacteria.**
- **(iii) they have a very low water content.**
- **2-** Why is an endospore called a resting structure?. Describe the most characteristics features of the endospore. Of what advantage is an endospore to a bacterial cell?.

References

1- Prescott, L. M., Harley, J. P. and Klein, D. A. (1993). Microbiology. 2nd edition. Wm. C. Brown Publishers, England.

2- Mansour, F.A. Principle of bacteriology. Mansoura University.

3-http:www.google.com (search for bacterial endospore).