

## CHAPTER 05: STUDY of the MAJOR BACTERIAL GROUPS

### 5.5. ACTINOMYCETES

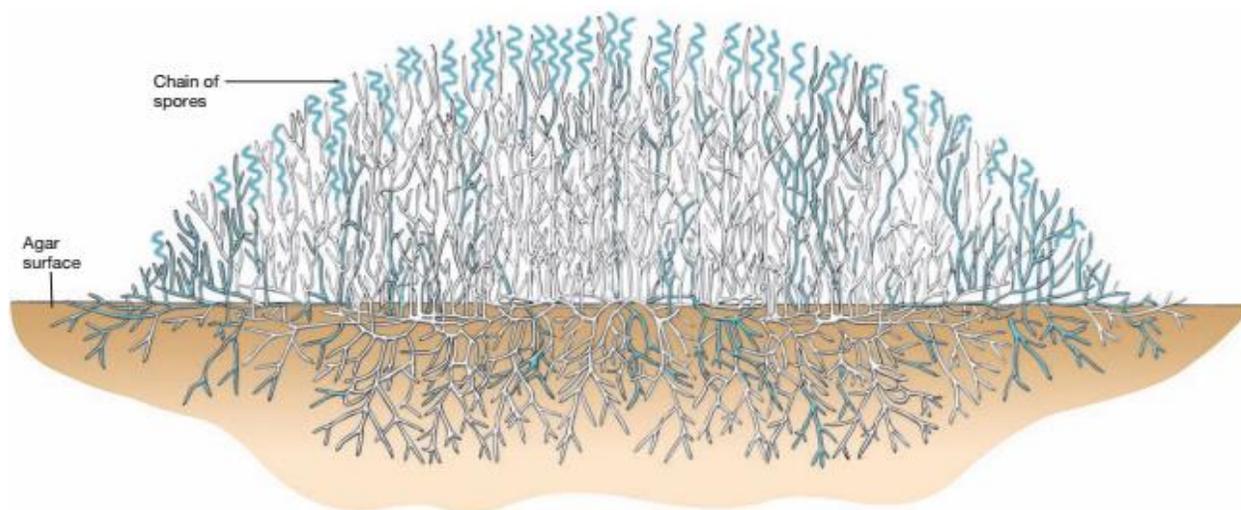
#### 1. General properties of the Actinomycetes

The actinomycetes are a fascinating group of microorganisms. They are the source of most of the antibiotics used in medicine today. They also produce metabolites that are used as anticancer drugs, antihelminthics (for instance ivermectin, which is given to dogs to prevent heart worm), and drugs that suppress the immune system in patients who have received organ transplants. This practical aspect of the actinomycetes is linked very closely to their mode of growth (complex life cycle). The life cycle of many actinomycetes includes the development of filamentous cells, called **hyphae**, and spores. When growing on a solid substratum such as soil or agar, the actinomycetes develop a branching network of hyphae. The hyphae grow both on the surface of the substratum and into it to form a dense mat of hyphae termed a **substrate mycelium** (Fig. 01). Septae usually divide the hyphae into long cells (20  $\mu$ m and longer) containing several nucleoids. In many actinomycetes, substrate hyphae differentiate into upwardly growing hyphae to form an **aerial mycelium** that extends above the substratum (Fig. 01). It is at this time that medically useful compounds are formed. Because the physiology of the actinomycete has switched from actively growing vegetative cells into this special cell type, these compounds are often called **secondary metabolites**.

The aerial hyphae form thin-walled spores upon septation (Fig. 01). These spores are considered exospores because they do not develop within a mother cell like the endospores of *Bacillus* and *Clostridium*. If the spores are located in a sporangium, they may be called **sporangiospores**. The spores can vary greatly in shape. Like spore formation in other bacteria, actinomycete sporulation is usually in response to nutrient deprivation. However, most actinomycete spores are not particularly heat resistant but withstand desiccation well, so they have considerable adaptive value. Most actinomycetes are not motile, and spores are dispersed by wind or adhering to animals; in this way they may find a new habitat.

Actinomycete cell wall composition varies greatly among different groups and is of considerable taxonomic importance. Four major cell wall types can be distinguished according to

three features of peptidoglycan composition and structure: the amino acid in tetrapeptide side chain position 3, the presence of glycine in interpeptide bridges, and peptidoglycan sugar content. Some other taxonomically valuable properties are the morphology and color of the mycelium and sporangia, the surface features and arrangement of spores, the percent G + C in DNA, the phospholipid composition of cell membranes, and spore heat resistance. Of course, 16S rRNA sequences has proven valuable.



**Figure 01:** An Actinomycete colony.

[The cross section of an actinomycete colony with living (blue-green) and dead (white) hyphae. The substrate mycelium and aerial mycelium with chains of spores are shown].

Actinomycetes also have great ecological significance. They are primarily soil inhabitants and are very widely distributed. They can degrade an enormous number and variety of organic compounds and are extremely important in the mineralization of organic matter. Although most actinomycetes are free-living microorganisms, a few are pathogens of humans, other animals, and some plants.

It has been clear for some time that some of the phenotypic traits traditionally used to determine actinomycete taxonomy do not always fit with 16S rRNA sequence data. Phylogenetic analyses based on 16S rRNA sequences are used to classify the high G + C Gram-positive bacteria. All are placed in the phylum *Actinobacteria*, large and very complex, which contains one class (*Actinobacteria*), five subclasses (e.g. *Actinobacteridae*), six orders (e.g. *Actinomycetales*), 14 suborders, and 44 families.

## 2. Classification and characteristics

**Phylum:** *Actinobacteria*

**Class:** *Actinobacteria*

**Subclass:** *Actinobacteridae*

**Order:** *Actinomycetales*

### 2.1. Suborder *Actinomycineae*

There is one family with five genera in the suborder *Actinomycineae*. Members of the genus *Actinomyces* are either straight or slightly curved rods that vary considerably in shape or slender filaments with true branching, Gram-positive and nonspore forming. They are either facultative or strict anaerobes that require CO<sub>2</sub> for optimal growth. The cell walls contain lysine but not diaminopimelic acid or glycine. *Actinomyces* species are normal inhabitants of mucosal surfaces of humans and other warm-blooded animals; the oral cavity is their preferred habitat. *A. bovis* causes lumpy jaw in cattle. *Actinomyces* is responsible for actinomycoses, ocular infections, and periodontal disease in humans. The most important human pathogen is *A. israelii*.

### 2.2. Suborder *Propionibacterineae*

This suborder contains two families and 14 genera. The genus *Propionibacterium* contains pleomorphic, nonmotile, nonsporing rods that are often club-shaped with one end tapered and the other end rounded. Cells also may be coccoid or branched. They can be single, in short chains, or in clumps. The genus is facultatively anaerobic or aerotolerant; lactate and sugars are fermented to produce large quantities of propionic and acetic acids, often carbon dioxide, and usually catalase positive. The genus is found growing on the skin and in the digestive tract of animals, and in dairy products such as cheese. *P. acnes* is involved with the development of body odor and acne vulgaris.

### 2.3. Suborder *Micrococcineae*

The suborder *Micrococcineae* has 14 families and a wide variety of genera. The best-known genera are *Micrococcus*, *Arthrobacter* and *Dermatophilus*. The genus *Micrococcus* contains aerobic, catalase-positive cocci that occur mainly in pairs, tetrads, or irregular clusters, usually nonmotile, does not undergo morphological differentiation. Micrococci colonies often are yellow, orange, or red. They are widespread in soil, water, and on mammalian skin, which may be their normal habitat.

The genus *Arthrobacter* contains aerobic, catalase-positive rods with respiratory metabolism and lysine in its peptidoglycan. Its most distinctive feature is a rod-coccus growth cycle (**figure 24.8**). When *Arthrobacter* grows in exponential phase, the bacteria are irregular, branched rods that may reproduce by a process called **snapping division**. As they enter stationary phase, the cells change to a coccoid form. Upon transfer to fresh medium, the coccoid cells differentiate to form actively growing rods. Although arthrobacters often are isolated from fish, sewage, and plant surfaces, their most important habitat is the soil. They are well adapted to this niche because they are very resistant to desiccation and nutrient deprivation. This genus is unusually flexible nutritionally and can even degrade some herbicides and pesticides.

#### **2.4. Suborder *Corynebacterineae***

This suborder contains seven families with several well-known genera. Three of the most important genera are *Corynebacterium*, *Mycobacterium*, and *Nocardia*.

The family *Corynebacteriaceae* has one genus, *Corynebacterium*, which contains aerobic and facultative, catalase-positive, straight to slightly curved rods, often with tapered ends. Club-shaped forms are also seen. The bacteria often remain partially attached after snapping division, resulting in angular arrangements of the cells, or a palisade arrangement. Some species are harmless saprophytes, many corynebacteria are plant or animal pathogens. For example, *C. diphtheriae* is the causative agent of diphtheria in humans.

The family *Mycobacteriaceae* contains the genus *Mycobacterium*, which is composed of slightly curved or straight rods that sometimes branch or form filaments. Mycobacterial filaments differ from those of actinomycetes in readily fragmenting into rods and coccoid bodies. They are aerobic and catalase positive. Mycobacteria grow very slowly and must be incubated for 2 to 40 days after inoculation of a solidified complex medium to form a visible colony. Their cell walls have a very high lipid content and contain waxes with 60 to 90 carbon **mycolic acids** (complex fatty acids). The presence of mycolic acids and other lipids outside the peptidoglycan layer makes mycobacteria **acid-fast**. Extraction of wall lipid with alkaline ethanol destroys acid-fastness.

*M. bovis* causes tuberculosis in cattle, other ruminants, and primates. Because this bacterium can produce tuberculosis in humans, dairy cattle are tested for the disease yearly; milk pasteurization kills the pathogen. Prior to widespread milk pasteurization, contaminated milk was

a problematic source of transmission. *M. leprae* causes leprosy which is mycobacterial human disease.

The family *Nocardiaceae* is composed of two genera, *Nocardia* and *Rhodococcus*. These bacteria develop a substrate mycelium that readily breaks into rods and coccoid elements. They also form an aerial mycelium that rises above the substratum and may produce conidia. Almost all are strict aerobes. Most species have peptidoglycan with *meso*-diaminopimelic acid and no peptide interbridge. The wall usually contains a carbohydrate composed of arabinose and galactose; mycolic acids are present in *Nocardia* and *Rhodococcus*.

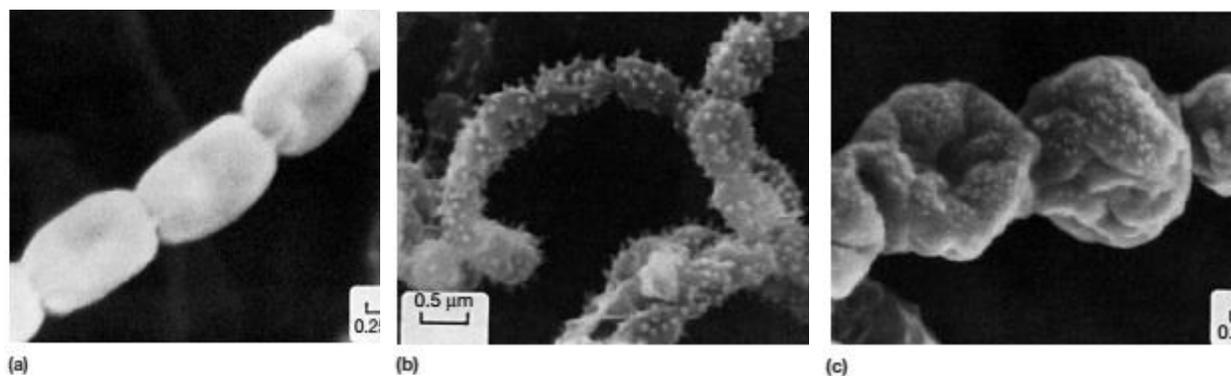
*Nocardia* is distributed worldwide in soil and aquatic habitats. Nocardiae are involved in the degradation of hydrocarbons and waxes and can contribute to the biodeterioration of rubber joints in water and sewage pipes. Although most are free-living saprophytes, some species, particularly *N. asteroides*, are opportunistic pathogens that cause nocardiosis in humans and other animals. The lungs are most often infected, but the central nervous system and other organs may be invaded.

*Rhodococcus* is widely distributed in soils and aquatic habitats. It is of considerable interest because members of the genus can degrade an enormous variety of molecules such as petroleum hydrocarbons, detergents, benzene, and various pesticides. It may be possible to use rhodococci to remove sulfur from fuels, thus reducing air pollution by sulfur oxide emissions.

## **2.5. Suborder *Streptomycineae***

The suborder *Streptomycineae* has only one family, *Streptomycetaceae*, and three genera, the most important of which is *Streptomyces*. These bacteria have aerial hyphae that divide in a single plane to form chains of 3 to 50 or more nonmotile spores with surface texture ranging from smooth to spiny and warty (Fig. 02). Filaments grow by tip extension rather than by fragmentation.

*Streptomyces* is a large genus; there are around 150 species. Members of the genus are strict aerobes, and form chains of nonmotile spores. The three to many spores in each chain are often pigmented and can be smooth, hairy, or spiny in texture. *Streptomyces* species are determined by means of a mixture of morphological and physiological characteristics, including the following: the color of the aerial and substrate mycelia, spore arrangement, surface features of individual spores, carbohydrate use, antibiotic production, melanin synthesis, nitrate reduction, and the hydrolysis of urea and hippuric acid.



**Figure 02:** Streptomyceete spore chains.

[(a) Smooth spores of *S. niveus*; scanning electron micrograph. (b) Spiny spores of *S. viridochromogenes*. (c) Warty spores of *S. pulcher*].

The natural habitat of most Streptomyces [Greek *Streptos*, bent or twisted, and *Myces*, fungus] is the soil, where they may constitute from 1 to 20% of the culturable population. In fact, the odor of moist earth is largely the result of streptomyceete production of volatile substances such as **geosmin**. Streptomyces play a major role in mineralization. They are flexible nutritionally and can aerobically degrade resistant substances such as pectin, lignin, chitin, keratin, latex, agar, and aromatic compounds. Although most streptomyces are nonpathogenic saprophytes, a few are associated with plant and animal diseases. *S. somaliensis* is the only streptomyceete known to be pathogenic to humans (**actinomycetoma**).

Streptomyces are best known for their synthesis of a vast array of antibiotics. The discovery of *S. griseus* that produces **streptomycin** by Stanley Waksman was an enormously important contribution to science and public health. Streptomycin was the first drug to effectively combat tuberculosis. The streptomyces have been found to produce over 10,000 bioactive compounds. Hundreds of these natural products are now used in medicine and industry; about two-thirds of the antimicrobial agents used in human and veterinary medicine are derived from the *Streptomyces*. Examples include amphotericin B, chloramphenicol, erythromycin, neomycin, nystatin, and tetracycline. Some *Streptomyces* species produce more than one antibiotic. Antibiotic-producing bacteria have genes that encode proteins that make them resistant to such compounds. The model species for research *Streptomyces coelicolor* produces four antibiotics.

## 2.6. Suborder Frankineae

The suborder *Frankineae* includes the type genus *Frankia*. It forms multilocular sporangia (Multilocular = have many cells or compartments.), characterized by clusters of spores when a

hypha divides both transversely and longitudinally. The G + C content varies from 57 to 75 mol%. *Frankia* forms nonmotile spores in a sporogenous body. It grows in symbiotic association with the roots of at least eight families of higher nonleguminous plants (e.g., alder trees) and is a microaerophile that can fix atmospheric nitrogen.

The roots of infected plants develop nodules that fix nitrogen so efficiently that a plant such as an alder can grow in the absence of combined nitrogen (e.g., NO<sub>3</sub><sup>-</sup>) when nodulated. Within the nodule cells, *Frankia* forms vesicles that may be the sites of nitrogen fixation (resembles that of *Rhizobium* in that it is oxygen sensitive and requires molybdenum and cobalt).

### **2.7. Suborder Micromonosporineae**

The suborder *Micromonosporineae* contains only one family, *Micromonosporaceae*. *Pilimelia* grows in association with keratin. *Micromonospora* actively degrades chitin and cellulose, and produces antibiotics such as gentamicin.

### **2.8. Suborder Streptosporangineae**

The suborder *Streptosporangineae* contains three families and 16 genera.