Section B and C

Volume-21

Contents

12. APPLIED BIOLOGY

A. MICROBIAL FERMENTATION AND PRODUCTION OF SMALL AND MACRO MOLECULES

FERMENTATION

It is necessary to define the term "fermentation," especially since this term is used extensively. Through the years, this word has gained new meanings, while retaining the old. Originally, fermentation referred to the bubbling observed when sugar and starchy materials underwent a transformation to yield alcoholic beverages. Later, this term was applied to the process in which alcohol was formed from sugar, regardless of whether the causative agent was or was not biological. Pasteur, considered fermentation to apply to those anaerobic reactions through which microorganisms obtained energy for growth in the absence of oxygen. Today, fermentation has a much broader meaning. It applies to both the aerobic and the anaerobic metabolic activities of microorganisms in which specific chemical changes are brought about in an organic substrate. In fact, from an industrial microbiology standpoint, the meaning is yet broader, and includes almost any process mediated by or involving microorganisms in which a product of economic value obtained.

1. ANTIBIOTICS AND ANTIBIOTIC FERMENTATION

Antibiotics are a special kind of chemotherapeutic agent, usually obtained from living organisms. The word antibiotic has come to refer to a metabolic product of one microorganism that in very small amounts is detrimental or inhibitory to other microorganisms. It has been known for many years that antagonisms can exist between microorganisms growing in a common environment. The term antibiosis was first defined by Vuillemin, in 1889, as a condition in which one creature destroys the life of another in order to sustain his own, the first being entirely active and the second entirely passive; one is in unrestricted opposition to the life of the other. However, it can be seen that this definition is not entirely compatible with the present-day use of the term antibiotics proposed by Waksman, in 1945, as applying to those chemical substances of microbial origin which in small amounts exert antimicrobial activity.

Antibiotics were known by their activities long before they were given the name by which we know them. Many years ago, the Chinese used mouldy soybean curd for the treatment of boils and controlled foot infections by wearing sandals furry with mould. In 1881, Tyndall reported that culture media cloudy with bacterial growth became elfin when mould grew on the

surface. Pasteur and Joubert found that pure cultures of Anthrax bacilli grew well in urine, but that, when certain other organisms were present, the Anthrax bacilli disappeared. This observation was related to that of Emmarich and Low, who demonstrated, in 1901, that when liquid cultures of *Pseudomonas aeruginosa* were injected into rabbits, the animals were protected against anthrax. They called this material pyocyanase, because, they thought its activity was due to enzymes from *Bacillus pyocyaneus, as P. aeruginosa* was then called.

An early clinical application of bacterial antagonism was the use of lactobacilli in the treatment of dysentery, as recommended by Metchnikoff, in 1899. This was an example of replacement therapy; *i.e.*, a harmless microbe was able to eliminate and replace one that could cause disease. Modern antibiosis is based not on replacement, but on utilization of an active inhibitory principle obtained from the antibiotic-producing microbes.

The first systematic search for and study of, antibiotics, made by Gratia and Dath around 1921, resulted in the discovery of actinomycetin in strains of actinomycetes, soil organisms that are representative of the group that has given us a number of antibiotics, since 1940. Actinomycetin was never used for the treatment of patients, but was used to lyse cultures of bacteria for the production of vaccines.

In 1929, Alexander Fleming noticed that an agar plate inoculated with *Staphylococcus aureus* had become contaminated with a mould and that the mould colony was surrounded by a clear zone, indicating inhibition of bacterial growth, or lysis of the bacteria. He was inspired to isolate and identify the mould and study its activities; but not until there was an urgent need for a better means of preventing death from infection of war wounds, was the importance of Fleming's observation realized. With the aid of many investigators in England and the United States, and at a great deal of expense, the inhibitory substance from Fleming's "contaminant mould" became a "miracle drug", the mould was identified as a *Penicillium sp.*, Fleming called the antibiotic penicillin.

In 1939, Rene Dubos isolated from New Jersey soil a culture of *Bacillus brevis* which produced a substance that killed many Gram-positive bacteria. The cell free extract produced from *B. brevis* by Dubos was found to contain two active principles, now known as gramicidin and tyrocidine. These successes were followed closely by the discovery of streptomycin by Salman Waksman and associates.

Several thousand antibiotic substances have been isolated and identified since 1940. Many of them are of no practical importance as yet, but a few have changed the entire concept of

chemotherapy. The popularity of antibiotics is due to their ability to destroy many kinds of pathogens and to their relatively nontoxic properties to the host when given systemically. Few developments in the field of medicine have had as dramatic effect as have antibiotics, in the treatment of microbial infections.

To be useful as chemotherapeutic agents, antibiotics must have the following qualities:

1. They should have the ability to destroy or inhibit many different species of pathogenic microorganisms. This is what is meant by a broad-spectrum antibiotic.

2. They should prevent the ready development of resistant forms of the parasites.

3. They should not produce undesirable side effects in the host, such as sensitivity or allergic reactions, nerve damage, or irritation of the kidneys and gastrointestinal tract.

4. They should not eliminate the normal microbial flora of the host; because, doing so may upset the "balance of nature" and permit normally nonpathogenic microbes, or particularly pathogenic forms normally restrained by the usual flora, to establish a new infection. The broad spectrum antibiotics, for example, may eliminate the normal bacterial flora, but not Monilia from the intestinal tract. Under these conditions the Monilia may establish an infection that is not controlled by antibiotic therapy.

Antibiotics can be classified in several ways. For example, some are bactericidal and others are bacteriostatic. They may be grouped on the basis of chemical structure. A third way of classifying antibiotics is on the basis of their mode of action that is the manner in which they manifest their damage upon microbial cells. Our discussion in this section will be organized on the basis of this latter manner of grouping antibiotics, i.e., their mode of action.

The major points of attack of antibiotics on microorganisms include:

1. Inhibition of cell wall synthesis,

2. Damage to the cytoplasmic membrane.

3. Inhibition of nucleic acid and protein synthesis and

4. Inhibition of specific enzyme systems.

Among the antibiotics whose antimicrobial activity is expressed by inhibition of the biosynthesis of the peptidoglycan of cell wall structure are the penicillins, cephalosporins, cydoserine, vancomycin, and bacitracin.

The substance that gives rigidity to the cell wall is the peptidoglycan. The structure of

this compound is essentially that of a series of strands (polymers with repeating units of N-acetylglucosamine and N-acetylmuramic acid) that are cross-linked with small peptides, with a frequency and in a manner that imparts considerable rigidity in cell wall. It is a protective covering for the bacterial cell.

The biosynthesis of peptidoglycan involves numerous steps. Interference with any step in the sequence may inhibit cell wall synthesis and result in the inability of the bacterium to survive, because of the absence of a protective covering (cell wall).

Early experimental evidence suggested that some antibiotics exert their antimicrobial effect by inhibiting biosynthesis of the peptidoglycan polymer, resulting in the inhibition of cell wall formation. Subsequent research identified the sequence of reactions in the biosynthetic pathway of the peptidoglycan and demonstrated that antibiotics like penicillin inhibited its formation. Some experimental observations that led to this conclusion can be summarized as follows:

1. Bacterial cells, susceptible to penicillin, can be protected from destruction if the medium in which they are exposed is of high osmotic pressure. The high osmotic pressure prevents the cells from bursting. Rod shaped cells become spherical; because, they lack the cell structure which imparts shape. These cells without cell walls are called spheroplasts**.**

2. Some species of bacteria, such as the mycoplasmas lack the peptidoglycan structure and are not inhibited by penicillin.

3. Concentrations of penicillin below that, which kill susceptible bacteria, result in the accumulation of compounds that are precursors to peptidoglycan formation.

The first of the modern antibiotics, and still one of the most useful, penicillin is produced by *Penicillium notatum; Penicillium chrysogenum,* and by other species of moulds. As previously noted, the first of these was isolated by Fleming, in 1929, when he found it as a contaminant on a culture plate. Florey and his associates at Oxford University, isolated the active ingredient and used the crude material clinically, in 1940. Penicillin is selective for Gram-positive bacteria, some spirochetes, and the Gram-negative diplococci (*Neisseria*). Although it is rarely toxic in human patients, it may give rise to sensitivity reactions which vary from a mild skin reaction to severe anaphylaxis.

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