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An Analysis on Concept and Strategies of Production of Antibiotic

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Abstract – Antibiotics are substances are obtained from bacteria and fungi. Antibiotics are used for many different purposes. The most important of these uses is as drugs to fight various diseases caused by harmful microorganisms. Use of antibiotics has now made it possible to treat many diseases that were fatal prior to development of antibiotics. A few are used to treat certain cancers.

Antibiotics cure diseases by their property of being selectively toxic to microorganisms. When administered to a patient, they damage certain types of cells in the patient's body, but do not damage others. Antibiotics used as medicines are harmful to the cells of disease-causing microorganisms. Such antibiotics are used to treat a variety of bacterial diseases. A small number of antibiotics have also been developed to attack human cells for treatment of cancer. They are able to cure cancer by only damaging cells that are in the process of dividing.

In common usage, an antibiotic is a substance or compound that kills or inhibits the growth of bacteria. Antibiotics belong to the group of antimicrobial compounds, i.e., those for treating infections caused by microorganisms, including viruses, fungi, and protozoa. The term "antibiotic" was coined by Selman Waksman in 1942 to describe any substance produced by a micro-organism that is antagonistic to the growth of other micro-organisms in high dilution.

This original definition excluded naturally occurring substances, such as gastric juice and hydrogen peroxide (they kill bacteria but are not produced by micro-organisms), and also excluded synthetic compounds such as the sulfonamides.

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INTRODUCTION

Although Fleming discovers penicillin in 1929, large scale processes for its production were not realized until the early 1940s. The treatment of wartime casualties became a priority and the work of Florey realized the potential of this drug in the battlefield. Initially penicillin was produced in milk bottles because the technology existed for the handling and filling of these vessels.

The early success in isolating a medically useful compound from a microorganism lead to the massive ongoing hunt for antibiotics that continues to the present day. Today, over 5000 compounds have been isolated; by far the largest proportion has been isolated from Streptomyces cultures.

Antibiotics are produced generally as a result of some environmental stress that triggers a metabolic response. Antibiotics are produced as a defense mechanism to prevent the proliferation of other organisms when environmental conditions are challenging. Of this only a relatively small number of compounds have been commercially successful in therapy, with hundreds of tons of compound being produced each year by fermentation. Penicillins and cephalosporin's account for approximately 70% of market share. These are used either in their natural form or as semi synthetic derivatives.

Culture Preservation-The preserved culture is a valuable asset and as little should be used as possible to initiate the process. Generally this preserved stock is in the form of inert spores. Spores can be stored in dry soil, lyophilised or frozen in a preservation solution.

Production Strain-A high yielding strain is a prerequisite of any good antibiotic producing process. Continuous modification of process conditions will result in steady increases in yield however; large steps forward generally result from the introduction of a superior strain. Such strains are generally obtained through genetic manipulation. Random mutagenesis and subsequent screening are key strategies in strain yield improvement.

Scale Up-Scale up is an important part of many cell driven processes and no more so than in antibiotic

fermentations. Starting off with a small volume of a preserved culture, it is not feasible to inoculate your production fermenter immediately with this material. It is essential to amplify the starting innoculum through a series of ever increasing volumes until enough material exists to innoculate the production system.

A whole family of production media is required for an antibiotic producing process. Each medium is designed to support a particular stage of the process Media designed for the early stage of the process will be generally focused on achieving optimal spore germination and strong vegetative growth. As antibiotics are secondary metabolites, the final production medium will be focused on the over production of the antibiotic often to the detriment of growth.

Most media used for production are complex in nature. Although many organisms will produce their indigenous compound on chemically defined media, complex media are cheaper to formulate and have higher yields and productivities. As with any general growth media it must supply all the basic constituents for growth/product formation.

HISTORY

The search for antibiotics began late in the 17th century with the growing acceptance of the germ theory of disease, a theory that linked bacteria and other microbes to the causation of a variety of ailments. This resulted in scientists putting a lot of time and effort into searching for drugs that would kill such pathogenic bacteria. The aim for the scientists was to discover a substance that would destroy the microbes without harming the person taking the drug. With extensive research this idea soon became a reality, and antibiotics were discovered. Initially they were produced at a small scale, although due to their huge success in a number of detrimental situations this changed considerably. The production of antibiotics once carried out in glass bottles changed drastically and huge steel fermenters were introduced. As time has passed by, the production process and the design of fermenters have progressed in a number of advantageous ways causing a substantial increase in antibiotic production.

The search for antibiotics began in the late 1800s. Ernest Duchesne, a French physician while working on his thesis noted that certain penicillin molds killed bacteria. He failed to bridge a connection between the fungus and a substance that had antibacterial properties. Within a few years Duchesne died, as did his research until a rediscovery in this area was made.

In the early 1920s, the British scientist Alexander Fleming documented that a certain product present in human tears had the ability to lyse bacterial cells. Fleming called his finding lysozyme and this became the first example of an antibacterial agent found in humans. Fleming's research continued and his next discovery in 1928 would change the world of medicine.

As Fleming was unable to purify the compound and also unable to carry out necessary clinical testing on animals and humans his work came to an end with his final work on penicillin published in 1931. In the late 1930's the progression of World War II increased the need for treating wound infections. All possible resources were pooled together so further investigation and study into purifying penicillin could be made. A hard working and dedicated team led by Howard Walter Florey succeeded with this task. They were able to produce useable quantities of the purified active ingredient, which were quickly tested on clinical cases. Animals and humans that were near-death with bacterial infections were miraculously cured with even small amounts of the drug in its crude form. Doctors were ecstatic with the discovery as this was a new reliable and rapid method for curing disease which before had been extremely difficult to cure, dreadful to tolerate and frequently fatal.

Cross-continent cooperation in the early 1940s resulted in the increased scale of penicillin production. At the time Britain had no facilities for mass production of the drug as almost all of its industrial capacity was dedicated to the war effort. Due to this the British and the USA worked closely to make penicillin a reality.

The organism is grown in the large fermenters and most antibiotics are produced by batched fermentations. In this case a volume of sterile medium in a vessel is inoculated and the broth is fermented for a period of time. The production vessel is carefully designed with a number of considerations. Homogeneity within the vessel must be obtained to ensure good bulk liquid mixing characteristics, high gas liquid mass transfer co-efficient must be attained and the vessel should have the ability to perform aseptically for prolonged periods of time. Good mixing and an adequate oxygen transfer rate are also desirable qualities of the fermenter. Next the tank is emptied and the proceeds extracted to yield the antibiotic. The quality of the fermented material can markedly affect the efficiency of the operations, for at the end of fermentation, the antibiotic concentration will never exceed 20g l –1 and may be as low as 0.5 g l –1.1 The point at which a fermentation process should be halted is an intricate decision and several important factors have to be considered. Usually a manufacturer will find it apt to harvest soon after the first signs of a faltering in the efficiency of conversion of sugar into the desired antibiotic.

The removal of cells is a crucial step in the extraction process. The cells are removed by filtration and this stage is carried out under sterile conditions to avoid contamination of filtrate with microorganisms that may lead to a serious or total loss of product.

ANTIBIOTIC PRODUCTION

Broadly defined, antibiotics include a chemically heterogeneous group of small organic molecules of microbial origin that, at low concentrations, are deleterious to the growth or metabolic activities of other microorganisms. That soil is rich in microorganisms capable of antibiotic synthesis is well accepted, but the frequency with which synthesis occurs at ecologically significant levels in nature has been much less clear. Over the past decade, however, genetic and molecular techniques, coupled with sensitive and bio analytical assays and equipment, have been applied to demonstrate conclusively that microorganisms synthesize a variety of antibiotics, even under field conditions, in the rhizosphere (that portion of the soil enriched in carbon and energy resources released by plant roots). These antibiotics can contribute to microbial competitiveness and the suppression of plant root pathogens, and the bacteria that produce them are therefore of considerable interest as a practical means of plant disease control.

When used together, the bio analytical and molecular approaches are complementary, allowing the detection and quantification of metabolites produced in situ as well as an evaluation of their activity, and hence their ecological significance. The direct detection of a metabolite provides irrefutable evidence that the genetic and physiological potentials for its synthesis have been met, and the amounts recovered are in part a function of the net rates of synthesis and turnover under particular experimental circumstances. Direct measurements are most informative when the identity and physical properties of the metabolite are known so that procedures for extraction can be optimized, and are of value in assessing the relative amounts present over a range of conditions or in monitoring persistence and dissemination in the environment.

Molecular approaches offer highly sensitive but indirect alternatives to the direct analysis of bioactive metabolites produced in situ. These techniques detect either the potential for synthesis as inferred from the presence or expression of biosynthetic genes, or an activity attributable to the presence of the metabolite itself. For example, introduced and indigenous antibiotic-producing strains can be detected and enumerated by using probes and primers based on unique DNA sequences within genes specific for antibiotic biosynthesis. Such sequences also have been applied to access novel biosynthetic genes directly from soil without the need for culturing. Reporter gene systems (described elsewhere in this volume) can be used to monitor the transcription of antibiotic biosynthesis genes expressed in situ. When the impact of metabolites on other organisms is of primary interest, as when antibiotic-producing agents are introduced for purposes of biological control, bioremediation, or bio fertilization, antibiotic-nonproducing mutant derivatives are indispensable in distinguishing between effects due specifically to the antibiotic and those attributable to other activities of the introduced agents

Antibiotics produced in situ adsorb rapidly to organic matter and charged groups on the surface of soil particles, and recovery declines continuously over time. The pH of soil and extractant solutions determines the charge of ionizable antibiotics, which in turn influences their solubility, affinity for soil colloids and organic matter, and uptake or diffusion into microorganisms and plants. Between pH 4.5 and 6.5, nonionic forms of organic acids and phenolic compounds are readily and irreversibly sorbed by soil organic matter or polymerized into humic substances. At pH values above the pKa (approximately pH 4.5 for phenolic acids), important charge interactions occur with components of the inorganic soil fraction. Interactions may be direct, as when hydroxy acid anions bind to positively charged metal oxides, or indirect, as when such acids bind to negatively charged surfaces through divalent cationic bridge molecules. Soils rich in hydroxy-Al and -Fe compounds have a high adsorption rate and capacity for carboxyl and phenolic hydroxyl groups, and some Mn2+-rich soils also have a high sorptive capacity for organic acid.

The processing of soil and plant tissue samples, including collecting, storing, and sieving, can affect antibiotic recovery. For plant-associated samples, it is important to note whether specific portions of the plant or root system are sampled, how much plant tissue and soil are present, and how they are separated. Quantitative variation can occur among replicate samples depending on whether macroscopic organic matter is distributed uniformly or removed by sieving.

Antibiotic-deficient mutants may arise spontaneously or be induced chemically, by ultraviolet irradiation, or with molecular genetics techniques. The latter approach is much preferred because the site of mutation can be localized to a specific biosynthetic gene. The strategy, which parallels Koch's postulates, consists of mutagenesis; phenotypic and/or genotypic characterization to identify antibiotic-deficient derivatives; complementation with wild-type DNA to restore antibiotic synthesis; and comparison of the activities of the mutant, wild type and restored phenotypes. A fifth step, in which the complementing gene is mutated, exchanged for the wild-type homologue, and shown to confer the mutant phenotype, is important to confirm the functional role of the mutated gene and to rule out the contribution of undetected second-site mutations, but often is over looked.

MODIFYING TECHNIQUES TO ANTIBIOTIC PRODUCTION

Most antibiotics are formed after the cell has ceased to divide. All such cultures have, therefore, a dual function—the reproduction of new cells and the synthesis of antibiotic by, the mature organism.

This process might be envisaged in a simple idealized surface culture as the formation of a mycelial mat and its subsequent elaboration to produce antibiotic. By definition, however, this ideal situation can never be realized. Establishment is not complete in a single reproductive phase; nor does all multiplication cease abruptly in favour of antibiotic production. Autolysing cells release nutrient; this supplements the unused original medium constituents and thus supports new growth to replace in part the spent mycelia.

It is clear, then, that even at this primitive stage of development a continuous process was going on in the culture, although it was not long maintained. Attempts to prevent this by admitting new nutrient under the pre-formed felt were only successful in the laboratory, for the mechanical difficulties in large-scale production were insuperable.

At this time, the limitations of surface culture were only too apparent. The use of thin layer culture meant that the production of adequate quantities of broth required acres of fermentation area. Moreover, the rate of production was limited by the slow diffusion of air through the mycelial mat and by the diffusion of solids upwards through the liquid layers. It was obvious that the organism should be more rapidly brought into contact with its oxygen and nutrient supplies.

The changing pattern of culture also affects one's approach to the selection of improved strains. When the course of the fermentation was almost entirely predetermined at inoculation, two criteria were applied, the ability to produce antibiotic more quickly and the ability to maintain the original rate longer, thus making better use of the medium. For example, most of the penicillin producing mutants selected by Farrell seems to have fallen into the second group. As continuous nutrition becomes a commonplace, it will clearly be more useful to select on a basis of better rate constants; although economic efficiency must not be neglected, this type of mutant is often more efficient under sub-optimal conditions. As well as leading to numerous developments in equipment design, continuous feeding techniques have also altered the logistics of factory operation.

CONCLUSION

Antibiotic resistance is a serious public health concern with economic, social and political implications. This study emphasises the need for comprehensive actions including information, training, legislation and education at all levels of drug delivery system to rationalize antibiotic use by improving prescribing pattern and creating awareness among consumers. This study recommends following preventive measures for prudent use of antibiotics. Antibiotics should not be used as growth promoters in animals and certain antibiotics should be used only for human medicine. All antibiotics should be sold only by prescription of autherised medical practitioners and availability of quality drug at affordable costs should be ensured.

This study emphasises the need for comprehensive actions including information, training, legislation and education at all levels of drug delivery system to rationalize antibiotic use by improving prescribing pattern and creating awareness among consumers. Proper disposal of pharmaceutical wastes is required to prevent the contamination of environment from pharmaceutical pollutants. Further study is essential concerning environmental impact of antibiotics.

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