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Penicillin production and history: An overview

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Abstract

Penicillin antibiotics were among the first drugs to be effective against different diseases. The discovery of penicillin and its medicinal uses was arguably the most important scientific discovery of the 20th century. As new ways were found to ensure that more penicillin was being produced and that the purification process was as effective and possible. Despite the expanding number of penicillin resistant bacteria, penicillin are used to treat a wide range of infections caused by certain susceptible bacteria Since then, the development of large scale production has allowed penicillin to be used whenever needed to kill off bacteria and prevent serious infection. The ability of some bacteria to now produce penicillinase to break down and render penicillin completely useless has come about due to the wide scale use of the drug and has therefore limited the effectiveness of penicillin as a clinical treatment.

Key words: Antibiotics, Historical Development, Penicillin, Resistance.

Introduction

History of penicillin

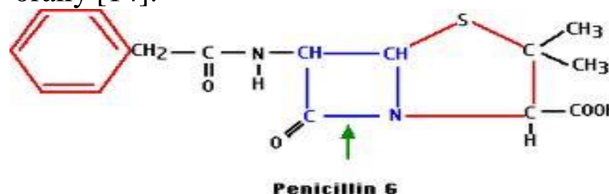
Alexander Fleming was the first to suggest that the Penicillium mould must secrete an antibacterial substance, the first to concentrate the active substance which he named penicillin, but he was not the first to use its properties as medicine. There are now more than 60 antibiotics, which are substances that are produced by microbes and that fight bacteria and fungi and other microbes harmful to humans. The word means against (anti) life (bio). Penicillin is obtained in a number of forms from Penicillium molds [2]. Penicillin is not a single compound but it is a group of closely related compounds, with all the same basic ring-like structure (a β-lactam) derived from two amino acids (valine and cysteine) via a tripeptide intermediate.

Bacteria reproduce by dividing to produce cells. They enlarge twice their size before the DNA copied. But when penicillin is present, the new cell wall can't be able to form. It doesn't harm old bacterial cell walls, but it stops new forming and

the bacteria can't reproduce, so the disease can't spread. Penicillin acts by blocking the activity of the enzyme transpeptidase, which cross connects long polymers of sugars that form the bacterial cell wall [1, 7]. The β-lactam ring on penicillin (see structure) irreversibly blocks the activity of the enzyme by covalently bonding. As a result, newly-formed cell walls will be structurally weak in some areas5, causing water to rush in and rupture the cell. The vast majority of antibiotics, even heavily modified, target the same cellular processes as their natural or synthetic predecessors [5]. The range of these targets is limited to the components of translational machinery, cell wall biosynthesis, DNA/RNA metabolism and some other cellular processes. There are two different categories of penicillin. Biosynthetic penicillin is natural bacteria) and an extended range of activity against some Gram-negative bacteria [10]. There are two different categories of penicillin. Biosynthetic penicillin is natural penicillin that is harvested from the mould itself through fermentation. The other form of penicillin is known as semi-synthetic. There are all kinds of what are called semi-synthetic derivatives of penicillin - like Penicillin V, Carbenicillin, Ampicillin, Oxacillin, Methicillin, etc. These compounds consist of the basic Penicillin structure, but have been purposefully modified chemically by removing the acyl group to leave 6-aminopenicillanic acid and then adding acyl groups that produce new properties. These modern semi-synthetic penicillin's have various specific properties such as resistance to stomach acids so that they can be taken orally, a degree of resistance to penicillinase (or β-lactamase) (a penicillin-destroying enzyme produced by some bacteria) and an extended range of activity against some Gram-negative bacteria [7, 8, 9]. Penicillin G (or benzyl penicillin) is the most widely used form and the same one we get in a shot (hypodermic) form.

Penicillin G

Benzyl penicillin (Penicillin G) is a narrow spectrum antibiotic used to treat infections caused by many bacteria. It is a natural penicillin antibiotic that is administered intravenously or intramuscularly. Penicillin G may also be used in some cases as prophylaxis and against different susceptible organisms [14, 15]. Penicillin G is not stable in the presence of acid. Since our stomach has a lot of hydrochloric acid in it (the pH can be around 2.0), if we ingest penicillin G, the compound would be destroyed in our stomach before it could be absorbed into the bloodstream, and would not be any good to us as a treatment for infection somewhere in our body8. It is for this reason that penicillin G must be taken by intramuscular injection - to get the compound in our bloodstream, which is not acidic at all. Many of the semi-synthetic penicillin's can be taken orally [14].



Penicillin G

Natural penicillin's are considered the drugs of choice for several infections caused by susceptible gram positive aerobic organisms such as *Streptococcus pneumonia*, groups A, B, C and G streptococci, non-enterococci group D streptococci, viridians group streptococci, and non-penicillinase producing *Staphylococcus* [6, 7, 9]. The history of penicillin is a fascinating reminder of how the most freak and seemingly random of occurrences can lead to very significant scientific discoveries. Also seeing as how the discovery of penicillin is arguably mains most important medical (if scientific) advance to date, some discussion of its origins are necessary. This history clearly displays that discovery in itself is not enough, there must also be a drive (in this case the huge numbers of soldiers dying from secondary infections in the second world war) to utilize this new discovery to its fullest. In 1928, while working in St. Mary's Hospital in London, bacteriologist Alexander Fleming was conducting research on the flu [2, 3]. He had been searching for antibacterial agents, influenced by his wartime experience. He had witnessed the deaths of many soldiers that died, not from the wounds they received during combat, but from secondary infections of those wounds [17]. While he was on holidays, a bit of blue-green mould had fallen into a discarded culture plate containing *Staphylococcus aureus*, forming a clear patch in the surrounding area. From this he could conclude that the mould was producing an antibiotic

substance [11]. He named the antibiotic penicillin, after the *Penicillium notatum* mould that produced it and 1929, he published the results of his investigations, noting that his discovery might have therapeutic value if it could be produced in quantity [14]. Unfortunately it couldn't, and it would be 10 years before another significant leap forward for penicillin would occur.

Albert Alexander, a 48-year-old London policeman had developed septicaemia as a result of a small cut on his face, when treated with penicillin Alexander began to recover within the day. However by 1941, it was acknowledged that penicillin was indeed a worthwhile drug and could save thousands of lives. In the same year Florey travelled to the United States (which at the time was still neutral) to continue his work with penicillin. Because the United States intended to enter into World War II in another few months the penicillin project, which became declared a war project, was given top priority (and funding) [14,17]. Florey and his team were able to use beer-brewing technology to produce the huge amounts of the moldy liquor needed for penicillin production. This underwent a slow purification process to produce the large amounts of clinically usable penicillin that became available for military use in early 1940's.

By late 1943, mass production of the drug had commenced and by the end of the war, many companies were manufacturing the drug, including the Merck, Squibb and Pfizer. In 1945 Fleming was awarded the Nobel Prize in Physiology and Medicine along with Florey and Chain [11].

Fermenters

A fermentor or fermentation chamber is a type of bioreactor for containing and controlling fermenter microorganisms, it is an economical route to important raw materials (ethanol, lactic acid) and fermented foods. An important part of any fermentation process (of which penicillin is an example) is the type and configuration of fermenter being used for the fermentation. Generally, the purpose of a fermenter is to provide a contained, controlled, homogenous environment in which the fermentation can proceed in a manner that is both safe and practical and which optimises the particular objectives of the fermentation. In the parameters, there is much more and huge range of variability and flexibility [7]. Flexibility is frequently a major objective in a lab. As reactors become larger and larger there is less and less room for error. When a reactor is being designed for a specific purpose there are a number of important parameters that will greatly affect the reactors process performance [15]. There are still are number of common desired features that are requirements (both functional and economic) for a good working reactor. Some parts are described below:

Mass Transfer Is seen when there is mixing between two components of varying concentrations. Mass Transfer processes are responsible for the movement of the dye through the water until equilibrium is reached. This is very important when we are attempting to grow aerobic microbes in media. Bulk Flow and Mixing are achieved by the impellers. Most reactors use the Rushton Flat-bladed disc turbine. Newtonian fluids show constant viscosity and linear shear rate against shear stress. Batch, Fed-Batch and Continuous Culture are terms used to describe how nutrients and substrate will be delivered to a culture in a reactor. Batch simply means that a fixed amount of substrate is added at the beginning of the process. Fed Batch means that substrate is added in small increments at various times in the fermentation. Steam is used to keep the reactor running aseptically. This is achieved because the reactor is designed as a pressure vessel and steam is sent through at a minimum temperature/pressure of 121O C/15 psi for 15-30 min.

Media for fermentation

Typically, all microorganisms require Hydrogen, Carbon, Sulphur Oxygen, and Nitrogen for cell growth and cell maintenance [10]. In many cases, microorganisms require small amounts of trace elements such as Cu, Mn and Co (this will frequently depend on the water source as most water sources contain small amounts of the elements) or growth factors such as vitamins or amino acids as well. Also, certain organisms such as *Penicillium chrysogenum* that produce antibiotics, enzymes or other secondary metabolites frequently require precursors like purine/pyrimidine bases or organic acids to

produce said metabolites. When performing any kind of fermentation, the selection of media is of critical importance to the overall performance of the fermentation [6, 7]. The aim of the media is to provide all the elements required for the synthesis of cell materials and the formation of the desired product. At the same time, the media must provide a favorable environment for the culture in question (an example of this would be control of pH by addition of calcium carbonate or inorganic phosphates). As well as this it must remain cost effective.

At the stage, it is necessary to give a brief discussion of the differences between primary and secondary metabolism. Primary metabolism is the metabolism of energy production for the cell and for its own biosynthesis [7]. Typically, in aerobic organisms (such as *Penicillium chrysogenum*) it involves the conversion of sugars such as glucose to pyruvic acid [2] and the production of energy via the TCA cycle. Secondary metabolism regards the production of metabolites that are not used in energy production for example penicillin from *Penicillium chrysogenum.* In this case the metabolite is being utilized as a defense mechanism against other microorganisms in the environment. In essence *Penicillium chrysogenum* can kill off the competition to allow itself to propagate efficiently. The last sentence is particularly important [6, 7]. Here it should be noted that these secondary metabolites are only produced in times of stress when resources are low and the organism much produce these compounds to kill off its competitors to allow it. To survive. It is these conditions that we wish to duplicate in order to achieve the maximum amount of product from our fermentation. This leads us onto the next point. Generally, this kind of fermentation is done in stages. In the first stage, primary metabolism will be emphasized. Media for this stage will typically be focused on achieving maximum growth and biomass production8. At this stage, Glucose (Starch) will usually be the primary source of carbohydrate for the culture. This is because this sugar is the most easily used for energy metabolism and thus gives the best yields in terms of growth.

Repression, where a secondary metabolite is inhibited by the presence of a more readily usable substrate). In the next stage, the production stage, we wish to do almost the exact opposite as the first stage. Once the desired biomass has been achieved, we wish to be travel the culture and induce the kind of stress conditions that trigger the production of the antibiotic. Limiting the amount of carbon and nitrogen available to the culture typically does this. Hence, the amount of carbon source that is added must be carefully controlled. In order to do this, lactose is frequently used as carbohydrate source in this part of the fermentation [6, 7, 8]. It is also very important that the nitrogen source is carefully controlled, as excess nitrogen will greatly inhibit antibiotic production. The solution to this is to use the fed-batch method to feed the culture. As stated above, this allows us to add the substrate to the reactor in small increments and to even change the substrate if we so desire. This is the best way to change from one production stage to another.

Processing and scale-up

This section deals with the various steps in a fermentation process, from the initial activation of a preserved culture right up to the full-scale industrial production of the antibiotic of interest. As can be imagined, there are numerous factors that must considered, each playing a different role at different stages in the fermentation. Almost all fermentations begin with the preserved culture18. This is a kind of stock of established culture from which other cultures are grown. Frequently, this will be a Stock that has been modified over time (genetically or not) to give the best possible yield of product and as such is generally very valuable. For this reason, it is often desirable to use as little of this stock as possible to initiate the fermentation process. Often the best way to preserve these cultures is in the form of inactive spores [18, 19]. This is because spores are very resistant structures that can survive heat, desiccation, low pH and mechanical forces that would typically kill a normal vegetative cell [7]. To increase their longevity, these spores are often kept in a cyropreservative fluid (e.g. Microbe®system) and frozen. In this way the cells can be preserved for many years.

To begin the fermentation process, a number of these spores will be introduced into a small (normally 250-500ml) conical flask where it will be incubated for several days. At this stage,

explosive growth is the most desired parameter and as such the medium in the flask will contain high amounts of easily utilizable carbon and nitrogen sources, Such as starch and corn-steep liquor. At this stage, the spores will begin to revive and form vegetative cells. Temperature is normally maintained at 23-28 (0) C and pH at 6.5, although there may be some changes made to facilitate optimum growth. The flask will often have baffles in it and be on a shaking apparatus to improve oxygen diffusion in the flask. Once the overall conditions for growth have been established and there is a viable Vegetative culture active inside the flask, it will be transferred to a 1 or 2 liter bench-top reactor. This reactor will be fitted with a number of instruments to allow the culture to be better observed than it was in the shake flask. Typical parameters observed include pH, temperature, and stirrer speed and dissolved oxygen concentration [7]. This allows tweaking of the process to occur and difficulties to be examined. For example, there may not be enough oxygen getting to the culture and hence it will be oxygen starved. Another example would be high shear-rate leading to cell damage. To resolve these problems, modifications will be made to the process and/or the reactor. The majority of optimization of the process occurs here in the bench-top reactors well as gathering of important information about the activity of the cells. At this point the cells should be showing filamentous morphology, as this is preferred for penicillin production. As before, cell growth is priority at this stage.

This reactor is often viewed as a type of prototype for the larger fermentations that follow. Once this has been successful the process is scaled-up again to a pilot-scale bioreactor [7, 8]. This reactor will be similar in design to the bench-top reactor except it will have a size of about 100-1000 liters. The aim here is to examine the effect of scale-up on the culture. At this stage, hopefully growth will continue as before, however, there are often sudden changes or loss in performance. This can be due to Changes in the morphology of the culture (remember *Penicillium chrysogenum* is a filamentous fungi and hence pseudo plastic) that may or may not be correctable. If the pilot-plant stage is successful then work can begin on an industrial scale operation. This is now very much an engineering problem. The reactor must be capable of running aseptically and the design must reflect safety and contamination requirements [12, 14]. At this stage the medium being added to the reactor will change. Now we wish to emphasis penicillin production over growth while maintaining a constant Volume. Carbon and nitrogen will be added sparingly alongside precursor molecules for penicillin fed-batch style. Another note is that the presence of penicillin in the reactor is itself inhibitory to the production of penicillin. Therefore, we must have an efficient method for the removal of this product and to maintain constant volume in the reactor. Other systems, such as cooling water supply, must also be considered [13]. If all this goes to plan, we should have fully functioning reactor that is production Penicillin ready for downstream processing [3]. From here it can be refined and packaged for marketing and distribution to a global market.

Resistance

Even before the overuse use of penicillin, some observations suggested that bacteria could destroy it by enzymatic degradation (Abraham and Chain, 1940). In general, though, the outlook was more or less optimistic. Methicillin-resistant *Staphylococcus sureus* (MRSA) is a type of bacteria that is resistant to certain antibiotics, including oxacillin, penicillin, methicillin and amoxicillin15. Staphylococcus infections including MRSA and many more, occur most frequently among persons in hospitals and healthcare facilities who have weakened immune systems [16]. *Staphylococcus aureus* is found on many individuals skin and causes no problems, there are a number of excellent reviews elsewhere describing a variety of antibiotic resistance mechanisms within the frames of the bullet-target concept, and these mechanisms can be classified as a target or bullet related14. Targets can be protected by modification (mutations making it insensitive to antibiotic action such as mutations in RNA polymerase conferring resistant to rifampin; (ii) modified by an enzyme (such as methylation of an adenine residue in 23S rRNA making it insensitive to macrolides); (iii) replaced and (iv) protected at cellular or population levels (formation of a protective barrier by secretion, for

example, of large amounts of exopolysaccharides). There is no tried and tested method for defeating organisms like MRSA, but as science advances, new ways of treating such resistant bacteria are being developed and will hopefully be effective in years to come [17].

Conclusion

The importance and value of antibiotics cannot be overestimated; we are totally dependent on them for the treatment of infectious diseases and they should never be considered mere commodities. In addition to their use in the treatment of infectious diseases and antibiotics are critical to the success of advanced surgical procedures including organ and prosthetic transplantation. There is no perfect antibiotic and once the most appropriate uses of any new compound are being identified, it is essential that prescription of the antibiotic be restricted to those uses. Various steps were taken so as to continue the use of antibiotics in different way. Although the penicillin's are still used clinically their value has been diminished by the widespread development.

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