Fermatix

BEAUTIFUL FERMENTATIONS

EXCELLENCE IN FERMENTATION

Volume 1
Fall 2024

Penicillin

Transition from Medieval to Modern Anti-Infectives Using Fermentation

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Foreword

The story of penicillin is the story of many things.

It is the story of profound human creativity and accomplishment.

It is the story of the human desire to improve upon Medieval therapeutics, the outcomes of which were uncertain at best.

It is the story of what good can come from the collaboration of an international group of scientists, industrialists, academic institutions, charities and governmental agencies.

It is the story of how it takes a team to be successful.

And, perhaps most poignantly to me, it is the story of how microbiology can make wonderfully complex and beautiful molecules — and how fermentation can be harnessed to produce them for the benefit of humanity.

Fermatix presents the story of Penicillin.

Early Approaches

Bacterial infections have plagued animals, including humans, since the dawn of time. Throughout that time, mankind has experimented with a wide variety of techniques to battle these infections. For example, "humorism" – the practice of balancing the "humors," including through bloodletting, emetics and purges – was practiced in the west for around 2,000 years and was recommended in medical texts well into the 1900's.

Both eastern and western medicine have a long history of using compounds containing heavy metals, such as mercury, bismuth and arsenic to fight bacterial infections and a host of other maladies. Mercury, as a therapeutic, was first widely used in the late 15th century to cure Syphilis, often in conjunction with opium elixir (paregoric).

Mercury chloride salts were also used to treat infection. For example, calomel ($HgCl_3$), which was referred to as mercurius dulcis (or sweet mercury), was widely used. But these chloride salts had an exceedingly small therapeutic dose window (likely the reason for the opium) and the side effects were dreadful, including salivation, gastroenteritis and severe impacts on the central nervous system (e.g., "mad hatter" syndrome, or the "Danbury Shakes").

Early in 18th century Europe, bismuth salts were identified as having medicinal properties and subsequently bismuth compounds began to be seen as a boon to humanity. For example, bismuth compounds were used to treat "cholera infantum". Prior to 1900, approximately 2 out of 10 children in America under the age of four died from this condition which is generally treatable with bismuth subsalicylate (the active ingredient in Pepto Bismol).

Meanwhile, arsenic was overcoming its history as a poison and proving valuable as a medicine. In 1907, Paul Ehrlich (who was awarded the Nobel Prize Physiology or Medicine in 1908 for different work) discovered an organoarsenic compound, his 606th arsenic preparation, that was to become Salvarsan. Shortly after, Ehrlich's lab invented Neosalvarsan,

a slightly less toxic compound in comparison to Salvarsan. Despite being toxic, both compounds were highly effective in the treatment of a variety of bacterial infections, and are considered the beginning of modern anti-infectives.

Although certain of these compounds are effective, and many are still used in limited circumstances today, they are generally toxic to both human and bacterial cells. Their non-selective nature, frequently resulting in serious side-effects, drove the search for alternatives.

The Rise of Sulfa Drugs

Prontosil, an azo dye derived from coal tar, ushered in the era of sulfonamide-based compounds and the industrial-scale production of anti-infectives.

In the 1930s, Gerhard Domagk, a scientist for IG Farben in Germany, was screening dyes and heavy metal compounds for potential therapeutic value, one of which was Prontosil. Domagk injected Prontosil into mice with streptococcal infections and all the mice survived. This outcome was especially surprising because Prontosil had proven inactive *in-vitro* but appeared to be extremely active in the live mice.

Gerhard Domagk
(1865—1964)

Domagk had actually made two amazing discoveries: 1) he discovered a prodrug (Prontosil), which is inactive *in-vitro*, and 2) an active anti-bacterial (sulfanilamide) that is produced *in-vivo* from Prontosil. It is believed that Domagk saved his own daughter's life with an injection of Prontosil.

Prontosil gets metabolized in the body to sulfanilamide, a much simpler compound, which is the active anti-infective. The success of Prontosil and the recognition of sulfanilamide's antibacterial properties led to an explosion in research, formulations and patents targeting many different medical conditions.

By 1937, over 100 companies were actively producing sulfonamide drugs. However, sulfonamides had several limitations, including:

- acting through a single mode of action
- a number of organisms were not affected by sulfonamides
- sulfonamide therapy resistance
- patient sensitivity and allergic reactions
- perceived dangers resulting from the "Sulfanilamide Disaster" and other events

Nevertheless, Gerhard Domagk won the Nobel Prize in Physiology or Medicine in 1939 for his discovery of Prontosil. Due to the war, the Nazi government forbid him from accepting the award at the time. Two years after the end of World War II, Damagk received his Nobel medallion.

The discovery and success of Protosil prompted researchers to accelerate their efforts to develop additional profitable, novel and patentable therapeutic compounds.

From The Lab Of Alexander Fleming

The story of the actual discovery of penicillin begins in the sloppy, disorganized lab of physician Alexander Fleming.

The Sulfanilamide Disaster

In 1937, S.E. Massengil Company of Bristol Tennessee produced a formulation of Prontosil that included a raspberry flavor and diethylene glycol. Apparently, it passed taste tests and was shipped all over the United States.
Unfortunately, diethylene glycol is tremendously poisonous—causing gastrointestinal, renal and neurological issues. By the time its toxic effects were known, over 100 people died.

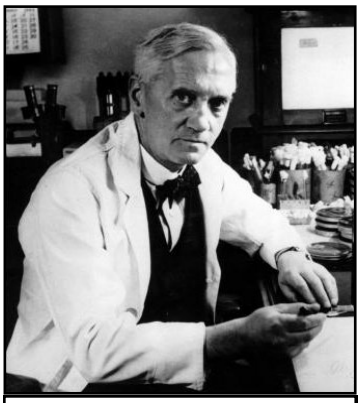
This incident led to the enactment of the 1938 Federal Food, Drug and Cosmetic Act, which formed the basis of modern drug regulation.

Fleming was well acquainted with deep wound anaerobic bacterial infections having served as a doctor in World War I and experienced the shortcomings of antiseptics in treating these frequently fatal infections.

In 1928, Fleming was studying the growth of various strains of *Staphylococcus aureus* at St. Mary's Hospital in London. After returning to his lab at St. Mary's Hospital following a vacation on September 3, 1928, he was sorting through plates of *Staphylococcus* bacteria and noticed one plate with a mycelium of green/blue mold.

The area around the mold was clear and devoid of

Staphylococcus colonies, while a short distance from the mold were colonies being lysed and then further out from the mold were normal colonies again. Fleming concluded that the mold was producing something that was killing the bacteria, which he called "mould juice."



Alexander Fleming
(1881-1955)

Fleming asked assistants Stuart Craddock and Frederick Ridley to purify the material – but the material proved unstable and only crude results were obtained. Many others also unsuccessfully attempted to purify the material.

Fleming published his findings in June 1929 in the British Journal of Experimental Pathology. In the article, Fleming correctly identified the Genus as *Penicillium*, but misidentified it as species rubrum. The blue/green mold was actually *Penicillium notatum*, a rather rare strain, as we would find out later.

In his article, Fleming named the "mould broth filtrate" penicillin. He noted that penicillin didn't appear particularly toxic and alluded to its potential therapeutic use toward the end of the article where he remarks on "…its possible use in the treatment of bacterial infections…".

To Oxford University

In 1939, Howard Florey and Ernst Chain of Oxford University began larger scale purification of penicillin, which culminated in the production of over 500 L per week of mold filtrate in any vessel available, such as food tins, bedpans, and milk bottles. Eventually, a custom bottle was produced, which saved space and a team of processors were employed at two pounds sterling per day. Large volumes of filtrate were extracted by biochemist Norman Heatley with amyl acetate and then purified using alumina in a new procedure called column chromatography.

In 1940, Florey showed that purified penicillin could protect mice infected with deadly Streptococci. In 1941, a policeman having a horrible infection was saved briefly by administering the purified penicillin, but he went on to expire when supplies ran out.

It was time to move to America where wartime manufacturing was producing machinery of all kinds and there was well-developed expertise in petrochemical processing techniques.

To The United States

With the help of the Rockefeller Foundation, Florey and Heatley travelled to America in the summer of 1941 to see if they could interest American pharmaceutical companies in the large-scale production of penicillin. Initially, the American pharmaceutical companies were not moved to action, likely because of the low reported yields from the fermentation. Eventually, Florey and Heatley made their way to Peoria, Illinois to the US Department of Agriculture's Northern Regional Research Laboratory (NRRL) because of its experienced fermentation personnel.

Robert Coghill took on the task at NRRL of increasing yields of penicillin. As fermentation experts frequently do, Coghill first looked at the nutrient package for the fermentation broth. Within weeks, the Coghill team was able to significantly increase yields by using lactose instead of sucrose as the carbon source.

A more important discovery was made only a short time thereafter when it was found that adding corn-steep liquor to the broth produced a 10-fold increase in the titer of penicillin.

Finally, the Coghill team was able to further increase yields by adding penicillin precursors, such as phenylacetic acid, to the fermentation broth.

For efficiency, the NRRL suggested submerged fermentation for large scale production. When first attempted, only small amounts of penicillin were produced with Florey's strain. As a result, researchers at the NRRL began trying to develop a more productive strain.

Under the direction of Kenneth Raper, many Penicillium strains were tested, and the best strain was found on a moldy cantaloupe in a fruit market in Peoria, Illinois.

The productivity of this strain was further increased by exposure to x-rays at Cold Spring Harbor in New York. These mutated strains were screened at the University of Minnesota and the most promising were forwarded to the University of Wisconsin Madison. At UW-Madison, Elizabeth McCoy identified the best mutant, called X-1612. Finally, botanists John Stauffer and Myron Backus took McCoy's strain and exposed it to ultraviolet light, resulting in the most productive wartime strain called Q-176.

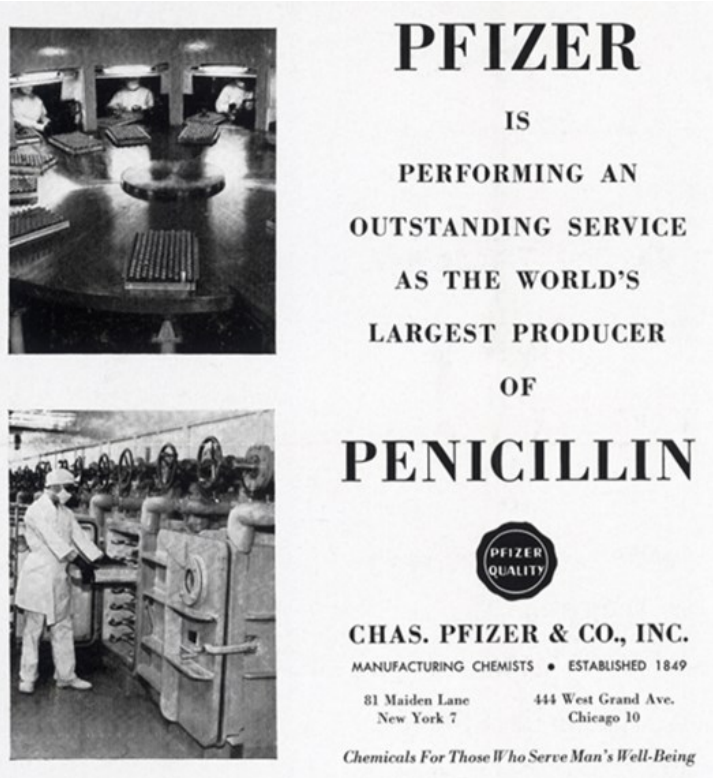
No patent applications were filed for this strain; rather, this strain was given freely to private companies in order to encourage industrial production.

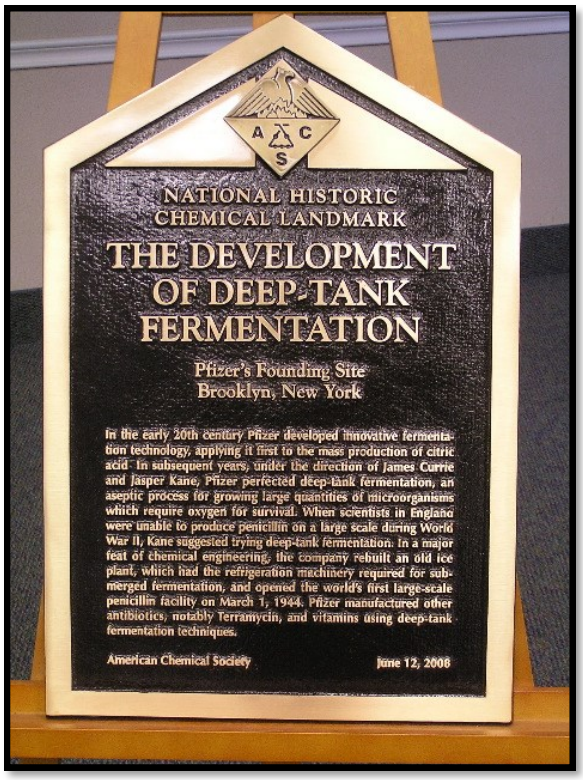
On Toward Commercial Production

Having been provided this more productive strain and with the promise of government support, Florey secured the interest of four pharmaceutical companies: Merck, Squibb, Lilly and Pfizer. Each company took a different approach, but fermentation ultimately proved the successful production method.

Various innovations were made quickly, including techniques for managing foam and delivering the vast volumes of air required for the fermentation, as well as techniques for purifying the resulting broth. In the final chapter of World War II, a small company based in New York called Pfizer beat the competition to commercially produce penicillin.

Pfizer was arguably the world's leading experts in industrial fermentation at the time, as they had years of experience producing citric acid (using *Aspergillis niger*), glucuronic acid, vitamin C, vitamins B2 and B12 by fermentation. They were able to leverage this experience to quickly develop a commercially viable production workflow for the new antibiotic.





On March 1, 1944, Pfizer opened the first commercial scale penicillin production plant in Brooklyn, New York utilizing submerged, deep tank fermentation. The plant cost Pfizer $3 million and used fourteen 7,500 liter fermentation vessels in production.

By D-Day (June 6, 1944), Pfizer had produced 2.3 million doses of penicillin. The American Chemical Society has designated development of the deep tank fermentation process by Pfizer as a National Historic Chemical Landmark.

The overall impact of this series of scientific and industrial accomplishments is difficult to quantify. For example, some estimate that over 200 million lives have been saved by penicillin since its discovery. Further, the techniques developed, and numerous industries spawned by this work have had tremendous global impact.

Modern fermentation had begun.

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At Fermatix, we're on a mission to fuel scientific exploration by providing high-quality enzymes at the lowest possible prices. We are committed to the development of robust manufacturing processes that facilitate the large-scale production of enzymes, allowing us to reduce your reagent costs. Why?

Because science should be accessible, and we're dedicated to making that a reality, one top-quality enzyme at a time.

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