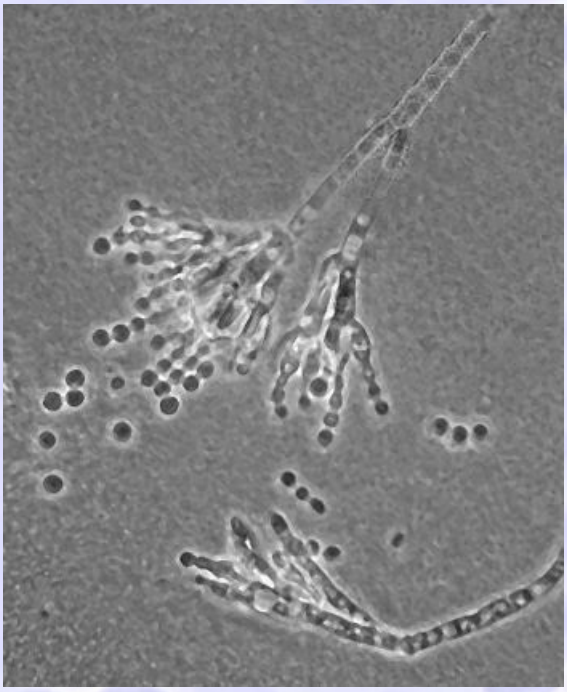
Tom Volk's Fungus of the Month for November 2003

This month's fungus is *Penicillium chrysogenum*, the source for penicillin--making it possible to have more veterans for Veteran's Day.

Please click TomVolkFungi.net for the rest of my pages on fungi



Penicillium chrysogenum (also known as *Penicillium notatum*) is the source for penicillin, the first antibiotic. Penicillin works against gram-positive bacteria, such as *Staphylococcus* and *Pneumococcus* by disrupting bacterial cell wall synthesis--crosslinking of the peptidoglycan polymers is prevented by inhibition of the enzyme transpeptidaase, causing the malformed cells walls to take on excess water, which causes them to burst (cell lysis).

The name *Penicillium* comes from the resemblance of the conidiophore of the fungus to a paintbrush-- penicillus is the Latin word for paintbrush. *Penicillium* is a member of the deuteromycetes, fungi with no known sexual state. Some species of *Penicillium* have an additional sexual state in the Ascomycota in the Eurotiales.

This month's fungus page honors Veteran's Day, celebrated in November in the USA. Although I am really a pacifist, I do like for soldiers and other people to survive bacterial infections. You can give penicillin credit for saving lives of soldiers with battle wounds, especially during the mid 20th century. Contrary to what you might think, most soldiers who died during World War I and WWII were not directly killed by gunfire or bombs, but rather died from the bacterial infections caused by those arms. For example, compare the healing rates of bone infections and compound fractures of wounded soldiers in WWI (25%) to those with similar wounds WWII, after penicillin became available (95%).

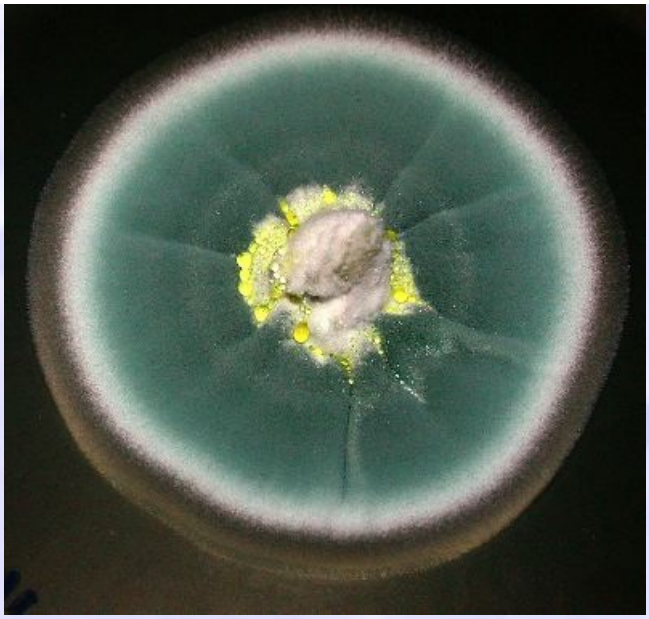
For those of you reading this from outside the United States, Veteran's Day is celebrated on November 11, the day World War I ended. Canada, the United Kingdom, Australia, and New Zealand celebrate this day as "Remembrance Day." This was known as Armistice Day until "the war to end all wars" was eclipsed by a much worse war, and one day to honor veterans was fittingly set aside for November 11. My own father fought in Japan during the later stages of WWII, and he said his life was extended many times by penicillin and other antibiotics. We are grateful to veterans for fighting in wars, but veterans should likewise be grateful for *Penicillium*'s production of penicillin, as well as the scientists who made that possible.

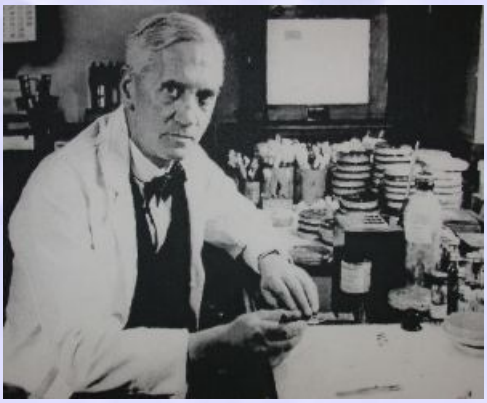
The discovery and development of Penicillin

As you have probably heard, penicillin was discovered quite by accident. Alexander Fleming was a Scottish-born microbiologist best known at that time for "painting" beautiful bucolic landscapes with bacteria in Petri dishes. He had also done some work during WWI with a bacteriolytic enzyme found in human tears. After the war he had a faculty position at St Mary's Hospital in London. Chance came a-knockin' when he went away for a weekend in 1928, but neglected to clean up his bacterial experiments with *Staphylococcus aureus*, leaving the dishes out on the lab bench (some accounts say in the sink). He returned after a long weekend and noticed that some mold was growing on one of the plates. He also noticed that there was a clearing in the lawn of bacteria around one of the molds. If this were the movies he would have shouted "Eureka!"-- but that's not how things happen in science. Most important discoveries in science don't start with "Eureka!" but with "hmmm, that's funny...." This discovery was no exception.

Discovery of penicillin was really a lucky set of coincidences. Fleming tried to repeat the inadvertent experiment, but with no success. As it turns out, it was unusually cold that summer, causing slow growth of both the bacteria and the fungus, which is necessary to see the inhibitory effect. Fleming

was unable to repeat the experiments right away under normal incubation conditions-- it was his colleague, Ronald Hare, who was able to reproduce the results by lowering the temperature. Not all strains of *P. chrysogenum* (=*P. notatum*) produce penicillin. The strain that fell onto Fleming's plates was a particularly good producer of penicillin. There is some evidence that the spores came from another lab in the same building in which he was working. So you might say that Fleming was only lucky. However, remember what Louis Pasteur said-- "Chance favors the prepared mind." The accidental contamination of bacterial plates by fungi is still very common, yet Fleming was the first to have the education and knowledge to realize that this could have some usefulness in treating disease.



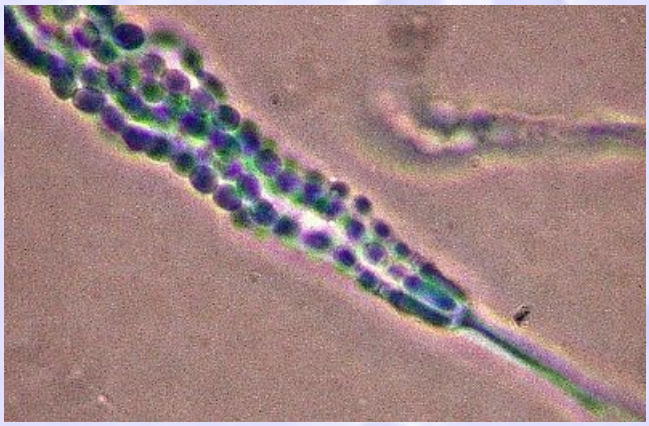


Fleming published his results in the 1929 paper "On the antibacterial action of cultures of a *Penicillium*, with special reference to their use in the isolation of *P. influenzae*" *in the* British Journal of Experimental Pathology 10:226-236). You can find the original paper online at this site and briefly summarized as follows. Fleming and his colleagues did a series of experiments that demonstrated the ability of extracts of the fungus to kill various gram-positive bacteria, including *Staphylococcus pyogenes, S. viridans, Micrococcus* spp., and a few others. Effectiveness against gram-negative bacteria (such as *Escherichia coli* and *Klebsiella pneumoniae*) was limited, with very high concentrations of penicillin needed to kill those organisms. We now know that a membrane protects those gram-negative bacteria against penicillin. Fleming *et al.* also performed careful experiments with injecting penicillin with no toxic effects into mice. They did not report on the cure of any bacterial diseases in mice, but speculated that penicillin might have some efficacy against infections by *Staphylococcus* and *Pneumococcus*. They identified the mold as *Penicillium rubrum*, which Charles Thom would identify (many years later) as *Penicillium notatum*. Later it was found that *P. notatum* was actually the same species as *Penicillium chrysogenum*, which, being an older name, became the correct name for the species. Fleming did not realize the practical importance of his discovery and went on to work on other projects.

[Much of the rest of this historical information comes from an excellent address by Kenneth B. Raper at the Third International Symposium on the Genetics of Industrial Microorganisms, Madison Wisconsin, reported as "The penicillin saga remembered." American Society for Microbiology News 44(12):645-653 1978.]

Further development of penicillin in Great Britain and the United States.

> "Penicillin would undoubtedly still have remained a fairly unknown substance, interesting to the bacteriologist but of no great practical importance, if it had not been taken up at the Pathological Institute at the venerable University of Oxford." -- Professor G. Liljestrand, introducing the winners of the 1945 Nobel Prize for Medicine.



After Fleming's paper was published, some work continued by Clutterbuck *et al.* in Pennsylvania, but Fleming's penicillin work went otherwise largely ignored, even by Fleming. At the 1939 International Microbiological Congress in New York when Charles Thom asked Fleming what had become of his penicillin, Fleming replied, "I forgot about that some years ago."

As World War II approached, the search for new drugs to combat bacterial infections of battle wounds became a primary focus of research, especially at Oxford university, where Howard Florey and

colleagues E.P. Abraham, Boris Chain, Norman Heatley, and Florey's wife Mary, who was a physician.

Penicillin was the prime candidate for an antibacterial antibiotic for the following reasons:

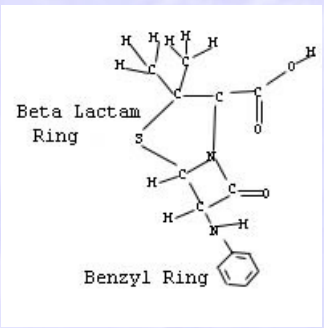
Penicillin kills gram-positive bacteria very well. On the plate to the left, *P. chrysogenum* (a descendent of Fleming's original strain) was inoculated into the center of a lawn of *Staphylococcus aureus*. Note the clear zone of inhibition of growth of *S. aureus*.
Penicillin causes no ill effects in humans and other animals, except for allergies in about 10% of humans. (We now know that penicillin allergies are usually caused by its binding to serum proteins, causing an IgE-mediated inflammation.)

However there were also obstacles to be overcome:

- Penicillin was unstable, especially at low and high pH.
- Penicillin was produced in small quantities by even the most prolific cultures.
- Penicillium grows well only in surface culture.

Florey and his colleagues at Oxford recognized the importance of Fleming's discovery and set out to solve these problems. They had already treated some patients with promising results. However the number of flasks they had to use to grow enough of the fungus to produce enough penicillin to treat even a single patient was prohibitively high. The first vessels they tried were some old-style bedpans, with a large surface area, a lid, and a side-arm for inoculation and withdrawal. It's always been unclear to me why a bedpan should have a side-arm-- maybe that's why they don't make them that way anymore. In fact by the time they discovered this as an ideal culture vessel they had already been replaced by more modern bedpans, and no more were available.

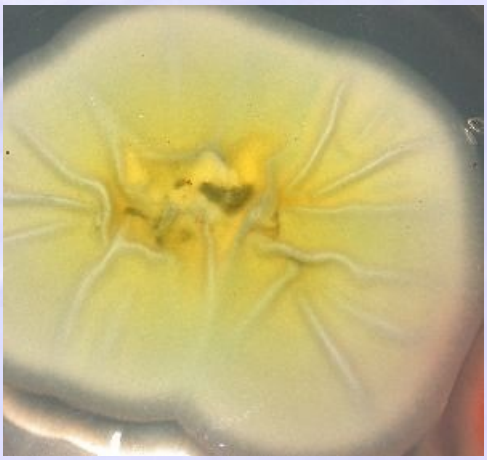
About that time the bombing of Britain made large scale production of penicillin there unlikely. Legend has it the researchers dusted the insides of their coats with spores of the fungus--in case something happened to destroy the lab. at least the fungus would survive. Florey and Heatley came to the United States in 1941 to try to interest some pharmaceutical companies in producing penicillin. They were not greeted with open arms, since penicillin production was in its very early stages of development. In particular, the production of penicillin was very low (4 units/ml where 1 unit=0.6micrograms), and the companies did not envision huge profits from such a small production. The National Academy of Sciences was consulted and sent them to Charles Thom, noted *Penicillium* expert (and coauthor of the monograph of *Penicillium* with Ken Raper). Thom sent them to the new Fermentation Division of the newly created Northern Regional Research Laboratory (NRRL) in Peoria, Illinois. It was there that most of the work on the industrialization of penicillin production was coordinated, with significant work by Ken Raper, and Drs. Moyer and Coghill.



By Christmas 1941, production was up to 40 units/ml due to modification of the culture medium. The main problem was that no derivative of the Fleming strain was ever found to produce penicillin in submerged culture or shaken flasks. The researchers surmised that they could find a strain of *P. chrysogenum* that would grow well in submerged culture. They asked everyone throughout the world to send in samples, moldy fruit,grains, and vegetables. Air Force and other military personnel were instructed to scoop up soil from exotic locations and have them sent to Peoria. The Peoria researchers even employed a young woman to scour the markets in Peoria for produce bearing blue-green molds. She became known as "Moldy Mary." However, after all that it was a moldy cantaloupe brought in by a Peoria housewife that proved to be the bonanza strain of *Penicillium chrysogenum*. In submerged culture the initial isolate produced 70-80 units/ml. By isolating single uninucleate conidia they found a mutant that yielded 250 units/ml.

Once word got around about this new wonder drug, home penicillin treatments were all the rage. Gauze floating on a nutrient broth grew mold, and the gauze was applied directly with a bandage to superficial wounds. Of course not all molds that people could grow were the right species of *Penicillium*. Moreover, even if the isolate is the correct species, not all strains even produce penicillin. Unfortunately this home remedy did not work.

Demands for penicillin were "unbelievable," so the War Production Board set up projects at other sites, including the University of Wisconsin-Madison, where J.F. Stauffer and Myron Backus tested thousands of ultraviolet light-induced mutations. (Incidentally, Ken Raper eventually became a professor of Botany and Bacteriology at UW-Madison, where I met him several times as a young graduate student. I even sat between him and Folke Skoog [discoverer of the plant hormone cytokinin] at a Badger football game!). Due to careful selection of mutants Backus and Stauffer were able to increase production from 250 to 900 units/ml. Additional mutagenesis on those strains resulted in a strain that could produce 2500 units/ml. Other universities became involved, including Stanford, Minnesota, and the Carnegie Institution in Cold Spring Harbor. It really became a group effort, with all groups making significant contributions to the development of penicillin. Industrial strains of *P. chrysogenum* now produce 50,000 units/ml, or about 30mg, significantly improved from the 4 units/ml starting point. Apparently, all the industrial strain used today are descendents of that single strain that came from the Peoria cantaloupe in 1943.



The Nobel Prize and the continuing importance of penicillin

For their work, Sir Alexander Fleming, Ernst Boris Chain, and Sir Howard Walter Florey were awarded the 1945 Nobel Prize in Medicine "for the discovery of penicillin and its curative effect in various infectious diseases." You can read more about their Nobel Prize here.

Here is part of the text of Fleming's Nobel acceptance speech:

"We all know that chance, fortune, fate or destiny - call it what you will-- has played a considerable part in many of the great discoveries in science. We do not know how many, for all scientists who have hit on something new have not disclosed exactly how it happened. We do know, though, that in many cases it was a chance observation, which took them into a track which eventually led to a real advance in knowledge or practice. This is especially true of the biological sciences for there we are dealing with living mechanisms about which there are enormous gaps in our knowledge."

Since WWII, many semisynthetic derivatives of penicillin have been made, including such familiar names as amoxicillin, ampicillin, methicillin, and augmentin. Penicillins paved the way for other antibiotics, such as cephalosporins, from the fungus *Acremonium*, and many others from Actinomycetes such as streptomycin from *Streptomyces*. Penicillin really changed the way doctors treat infections and diseases.

[Previous readers of this page will have seen *Penicillium notatum* as the name for this FotM, but thanks to an email from Keith Seifert of Agriculture Canada, I have updated this page to reflect the synonymy of the two *Penicillium species* and changed to the currently accepted name of *P. chrysogenum*. And they say the internet isn't peer-reviewed....]

If you have anything to add, or if you have corrections, comments, or recommendations for future FotM's (or maybe you'd like to be co-author of a FotM?), please write to me at volk.thom@uwlax.edu

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