How-To-Do-It

The Function of Penicillin in Nature and its Use in Medicine

A Laboratory Exercise

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By definition, an antibiotic is a chemical that is produced by a living microorganism and is harmful to other microorganisms. Prior to the discovery of antibiotics, it had been known for many years that antagonisms can exist between microorganisms living in the same 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" (Pelczar, Chan & Krieg 1986). Thus it can be seen that antibiosis is a natural phenomenon, and was first recognized as being so. It was not long, however, before attempts were made to exploit antibiosis for the treatment of infectious diseases. An early medical application of antibiosis was the attempted use of lactobacilli in the treatment of dysentery, as recommended by Metchnikoff in 1899 (Pelczar Chan & Krieg 1986). It was hoped that lactobacilli, which are bacteria that are harmless to human beings, would cause bacteria that cause dysentery to be eliminated from the digestive tract. Such attempts were not successful.

Modern exploitation of antibiosis is based not on the actual use of microorganisms to combat other microorganisms infecting the body, but on the use of antimicrobial chemical agents produced by microorganisms. These chemical agents are the weapons used by some species of microorganisms to combat other species of microorganisms in their natural habitat.

In 1929, Alexander Fleming reported that a culture of members of the bacterial genus *Staphylococcus* had become contaminated with a mold, and that the mold colony was surrounded by a clear zone in which there was little or no growth of bacteria, indicating that the mold was having a detrimental effect on the bacteria. Fleming stated:

> While working with staphylococcus variants a number of culture plates were set aside on the laboratory bench and examined from time to time. In the examinations these plates were necessarily exposed to the air and became contaminated with various micro-organisms. It was noticed that around a large colony of contaminating mould the staphylococcus colonies became transparent and were obviously undergoing lysis. Subcultures of this mould were made and experiments conducted with a view of ascertaining something of the properties of the bacteriolytic substance which had evidently been formed in the mould culture and which had diffused into the surrounding medium.

It was found that the contaminating mold was *Penicillium notatum,* and Fleming called the bacteriolytic substance which had been secreted from the mold into the bacterial growth medium *penicillin.* This discovery by Fleming opened the era of antibiotics (Bardell 1978). The discovery and development of penicillin for the treatment of human beings and domestic animals are among mankind's greatest achievements.

In the treatment of bacterial diseases the selection of the most effective antibiotic is crucial. The paper-disk method is the most commonly used technique for determining susceptibility of bacteria to antibiotics (Pelczar, Chan & Krieg 1986). Small paper disks impregnated with different antibiotics are placed on the surface of a solid growth medium that has been inoculated with bacteria. After a period for growth of the bacteria, the culture is observed for any zones of inhibition surrounding the disks. A zone of inhibition, a clear area, around a disk indicates that the microorganism was inhibited by the antibiotic, which had diffused into the growth medium from the disk. This procedure is a routine microbiology laboratory exercise. However, the exercise demonstrates only the medical use of antibiotics, and does not make the student aware of the source of antibiotics or the purpose of antibiotics in a natural environment. *Penicillium notatum* is easy to grow and work with in an ordinary biology laboratory, and doesn't require the facilities and special equipment of a microbiology teaching laboratory. Likewise, some of the common nonpathogenic bacteria can be grown and worked with easily. Therefore, general biology students can readily observe populations of molds and bacteria inhabiting a common environment and interacting with one another. Such an exercise is not only relevant for students with an interest in microbiology or medicine, but it has a broader manifestation, since it demonstrates a basic phenomenon of biology—competition among different species that have similar nutritional requirements and life-styles.

For the exercise reported herein, *Penicillium notatum, Staphylococcus epidermidis* and *Escherichia coli* were the organisms used. *Penicillium notatum* is a mold that normally inhabits soil, *Staphylococcus epidermidis* is a nonpathogenic gram-positive bacterium that normally inhabits the skin of human beings, and *Escherichia coli* is a nonpathogenic gram-negative bacterium that normally inhabits the intestinal tract of human beings. All three organisms grow well on brain-heart infusion agar, a medium frequently used for the growth of bacteria. The organisms and their growth medium are available from biological supply companies. Furthermore, all three organisms are approved for use by high school as well as college students. Ready-made medium can be obtained if an autoclave is not available for sterilization of dehydrated medium that needs to be reconstituted in distilled water. The exercise was first devised as an exercise in which students could duplicate Fleming's original observation, hence the reason for chosing a member of the genus *Staphylococcus.* The exercise was later extended to include a gram-negative bacterium; and

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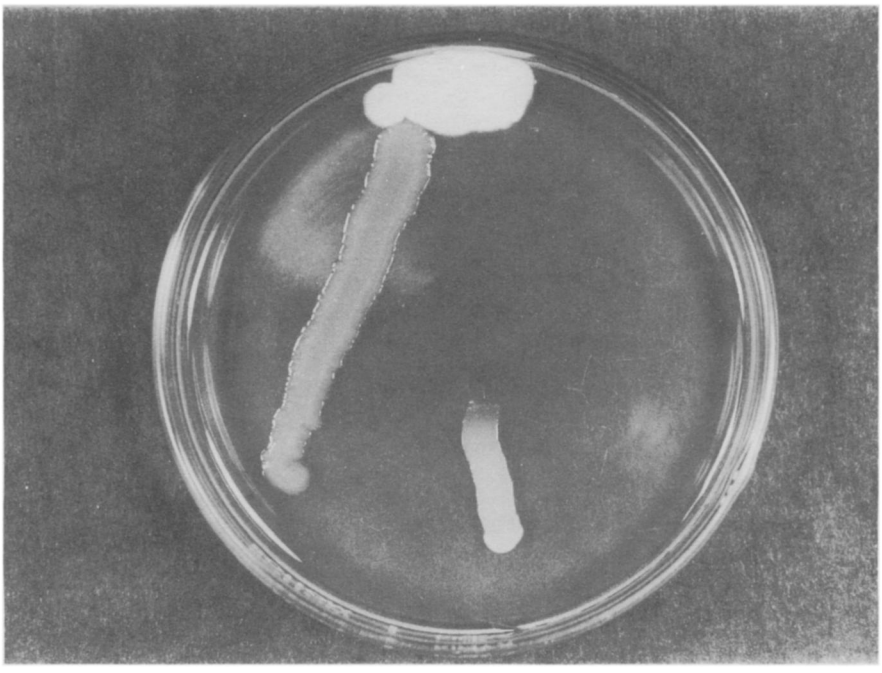

Figure 1. The effect of penicillin on bacteria. *Penicillium notatum*, a soil-dwelling mold, was inoculated at the edge of growth medium in a petri dish. After the development of a colony of the mold, the growth medium was streaked with bacteria from the edge of the mold to the opposite side of the dish. Penicillin produced by the mold and secreted into its environment prevented the growth of the gram-positive bacterium *Staphylococcus epidermidis*. This bacterium was streaked on the right side of the dish and no growth occured in the area where penicillin had diffused to from the mold colony. Growth of *Staphylococcus epidermidis* can be seen at a distance away from the mold where penicillin had not reached by diffusion. Penicillin had no effect on the gram-negative bacterium *Escherichia coli* streaked on the left side of the dish, and growth of this organism can be seen along the entire length of the streak line.

Escherichia coli was selected for convenience, as, like *Staphylococcus epidermidis*, it is associated with the human body and is harmless and easy to obtain.

Using an inoculating loop and aseptic technique, *Penicillium notatum* from a stock or purchased culture is inoculated onto brain-heart infusion agar in a petri dish. Inoculation is on the surface of the agar at the side of the dish and is restricted to a circular area about 1-2 cm in diameter; thus, after growth of a mold colony, there is still plenty of space for the two species of bacteria to grow. The inoculated dish is then left at room temperature (approximately 21°C) for 3 or 4 days, after which there should be a well established colony of *Penicillium notatum*. The dish is now ready to be inoculated with bacteria. Again, using an inoculating loop and aseptic technique, streak one of the bacterial species in a continuous line from close to the edge of the mold colony across to the other side of the dish. Then repeat the procedure with the other bacterial species. Take care that the mold colony is not touched by the inoculating loop when you streak the bacteria. In addition, care should be taken to ensure that the streaks of bacteria are far enough apart so that there is no cross contamination of the two bacterial species, and also far enough apart so that when the bacteria grow into visible colonies they do not grow into each other. The dish is then incubated at 37°C for 18-24 hours to allow for growth of the bacteria. If a 37°C incubator is not available, *Staphylococcus epidermidis* and *Escherichia coli* will grow at room temperature, but require a longer growth period.

Examination of the dish at the end of the bacterial growth period will reveal good growth of *Escherichia coli* along the entire streak line, whereas growth of *Staphylococcus epidermidis* occurs only at the distal end of the streak line (Figure 1). A bacterium cannot survive without its cell wall. There are considerable chemical differences between the cell walls of gram-negative and gram-positive bacteria. Penicillin is harmful to the gram-positive cell wall, but not to the gram-negative cell wall. Consequently, *Escherichia coli* was able to grow in the presence of penicillin, but growth of *Staphylococcus epidermidis* was prevented in the area in which penicillin had diffused to after its production and secretion by *Penicillium notatum*. These results demonstrate the effectiveness of penicillin for treating diseases caused by gram-positive bacteria. No antibiotic is effective against all bacteria, and the results with *Escherichia coli* demonstrate that fact as well.

This laboratory exercise has an intrinsic control; you can see that *Staphylococcus epidermidis* was viable and conditions for its growth were met from the growth of the organism at a distance away from *Penicillium notatum* where penicillin had not reached by diffusion. If the colony of *Penicillium notatum* is large at the time when the bacteria are inoculated, enough penicillin will have been produced to diffuse throughout the growth medium in the dish, and then there will be no growth at all of *Staphylococcus epidermidis*. In that case it would be necessary to have a control for the viability and growth of *Staphylococcus epidermidis*.

The ability of *Penicillium notatum* to produce penicillin did not evolve for the purpose of being recognized by Fleming as a possible antimicrobial agent for the benefit of human beings. It evolved as a mechanism by which *Penicillium notatum* competes with other species of soil-dwelling microorganisms. Soil is the natural habitat for numerous species of microorganisms. Indeed, microorganisms are essential for the production of soil and also for making it fertile for plant growth. In a biological community no species lives in isolation from other species. Competition occurs when two or more species utilize a common and limited resource such as food. A variety of different mechanisms that allow competition between one species and another are seen in plants and animals and microorganisms. Antibiosis is a much used mechanism for competition among bacteria and microfungi that inhabit soil. Since competition by antibiosis is common among soil microorganisms, most of the many different antibiotics used in medicine are derived from soil microorganisms. Another example, in addition to penicillin produced by *Penicillium notatum*, is streptomycin produced by the soil-dwelling bacterium *Streptomyces griseus*.

References

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