

Testing Soil Microbes for Antibiotic Production

Lauren Atkinson and Barbara Murdoch

Dept. of Biology, Eastern Connecticut State University,
Science Building, Room 353, 83 Windham St., Willimantic, CT 06226

Abstract

The discovery of antibiotics revolutionized medicine by making dangerous bacterial infections and diseases easily treatable. Life expectancies climbed in part due to the ease of treatment for diseases such as tuberculosis and pneumonia, diseases that for centuries were practically a death sentence. Furthermore, antibiotics decreased the risk of post-surgery infections, allowing doctors to develop more sophisticated surgical procedures that extended the average human life expectancy even further. However, these advances are at risk as the pathogens that antibiotics once easily controlled are rapidly evolving resistance to antibiotics due to their widespread overuse and misuse. Antibiotic-resistant infections exact a high price both to human life and to the economy, and experts in medicine, microbiology, economics, and other fields predict that these costs will only increase unless immediate action is taken. This study's purpose is to isolate antibiotic compounds that may be used to create antibiotics to which bacterial pathogens are not resistant. This research cultured on various media the bacteria in soil samples from Church Farm, Ashford, CT. This study utilized morphology to characterize the bacterial isolates and tested them for antibiotic activity against non-pathogenic bacteria.

Introduction

The discovery of the antibiotic properties of penicillin by Alexander Fleming in 1928 ushered in a golden era of medicine, an era in which previously deadly bacterial diseases and infections were easily treatable (Ventola, 2015). Surgeons made incredible advances as the risk of post-surgery infection plummeted. Today, sophisticated procedures such as organ transplants and joint replacement surgeries have become routine, in stark contrast to the pre-antibiotic era when even minor surgeries were risky.

However, this golden age of medicine is drawing to an end as pathogens that antibiotics once easily controlled are rapidly evolving resistance to antibiotics. Antibiotic resistance, the ability of bacteria to grow and thrive even in the presence of antibiotics, is in part due to the widespread overuse and misuse of antibiotics. The diminished effectiveness of antibiotics hampers efforts to control infectious diseases and to address complications among vulnerable patients such as premature infants, the elderly and chemotherapy patients. In 2016, Dr. Margaret Chan of the World Health Organization warned the UN General Assembly that without immediate action, we face the end of modern medicine as we know it (WHO, 2016).

The societal and economic costs of antibiotic resistance are crushing. In the

United States alone, over 2 million infections and 23,000 deaths are directly caused by antibiotic-resistant pathogens (CDC, 2013). Antibiotic-resistant infections cost the United States over \$20 billion in health care costs and over \$35 billion in lost productivity (Ventola, 2015). If the rate of development for both resistance and new effective treatments remain the same, antimicrobial resistance will claim the lives of 10 million per year and cost the world economy over \$100 trillion by 2050 (O'Neill, 2014). While this estimate includes the effects of antiviral and antifungal resistance, antibiotic resistance is the main driver.

Most of the recommendations from scientists for addressing antibiotic resistance fall into two main categories: decreasing the inappropriate use of antibiotics and encouraging research to discover new drugs. Much of the research devoted to discovering new antibiotic compounds focuses on soil bacteria, because most of the antibiotic compounds discovered to date were isolated from soil bacteria. Soil is rich in microbial life, with experts estimating that a single gram may contain 1 billion bacterial cells and 8 million different bacterial species (Gans et. al., 2005; Schloss and Handelsman, 2006). In this study, the bacteria in soil samples from two sites were tested for antibiotic activity.

Materials and Methods

Soil Collection

Soil samples were collected in February 2016 from two sites at Church Farm, Ashford, CT. The air temperature was between 13-15°C. The first sample (Site 1) was collected at a depth of 1.2 cm from a dry and sunny area underneath an apple tree in a field of grasses. The soil temperature was 5.5°C, and the soil type was sandy loam. The second sample (Site 2) was collected at a depth of 4.6 cm from the edge of a deciduous forest with patches of melting snow. The soil sample had to be taken at a greater depth because of the amount of leaf litter and ice mixed with the soil. The soil temperature was 1.3°C, and the

soil type was sandy clay loam.

Culturing Techniques

For each sample, 1g of soil was diluted with 9 ml of distilled water to make a 1:10 stock solution. Four serial 1:10 solutions were made for each sample. The last three serial solutions derived from Site 1 were streaked onto two different growth plates - potato dextrose agar (PDA) and lysogeny broth (LB). The last three serial dilutions derived from Site 2 were streaked on LB and tryptic soy agar (TSA). The plates were incubated at room temperature for one week, after which the total number of colonies on each plate was counted and their morphologies were recorded. The

colonies were subcultured on the same growth medium as the primary culture, for one week to obtain colonial isolates. Four of the original plates grew unwanted fungal colonies. To avoid culturing fungi, some of the bacterial colonies were not subcultured since their growth overlapped with that of the fungi.

Testing for Antibiotic Production

The colonial isolates were plated against safe relatives of the ESKAPE pathogens using the patch/patch method to test for antibiotic production. ESKAPE pathogens are the six bacterial pathogens that cause the majority of hospital-contracted infections and

most antibiotic-resistant infections (Rice, 2008). For the patch/patch test, a safe relative of these bacteria is patched at the center of a petri dish and the bacterial isolates being tested for antibiotic activity are patched at the edges of the plate. The bacteria from Sites 1 and 2 cultured on LB media were tested against *Escherichia coli*. Bacteria from Site 1 cultured on PDA media were tested against *Staphylococcus epidermidis*. Bacteria from Site 2 cultured on TSA media were tested against *Bacillus subtilis*. After incubation for one week at room temperature, the plates were examined for zones of inhibition, areas where bacterial growth was deterred, indicating antibiotic production.

Results

We examined the soil samples from two different sites at Church Farm, Ashford, CT (Fig. 1 A-D) to determine if bacteria capable of producing antibiotics were present. To determine this, we isolated bacteria from the samples and tested them for antibiotic activity.

Formerly a field, Site 1 is an open area dominated by grasses (Fig. 1A). It is a sunny, well-drained area. Site 2 is a low-lying, shady area dominated by shrubs and trees at the edge of a woodland bordering the field (Fig 1B). The ground is covered with leaf litter that retain moisture. Soil from Site 1 was significantly warmer than that of Site 2 (see materials and methods), due to the amount of sunlight received. The sites were selected because while they are both nutrient-rich environments, they are very different habitats, supporting different vegetation types.

After one week of incubation at room temperature, on the primary 12 plates we found a total of 179 colonies. The plates with the most colonies on them (Site 1 LB 1:100, Site 1 PDA 1:100, Site 2 LB 1:100, and Site 2 TSA 1:100) had a high percentage of fungal colonies (data not shown). The fungi and the bacterial colonies touching the fungi were avoided when subculturing the colonies.



Figure 1. The Church Farm Study Site, Ashford, CT. A) Site 1 – Apple tree in a field of grasses. B) Site 2 – Snowy forest. C,D) Location and aerial view of Church Farm.

A detailed account of the number of colonies that were transferred to master plates for storage (Fig 2) and subsequently tested for antibiotic production is presented in Table 1. A total of 62 colonies were subcultured and tested for antibiotic production, that would be indicated by inhibitory action against the growth of the following bacterial strains - *S. epidermis*, *B. subtilis*, and *E. coli* (Fig 3A-G).

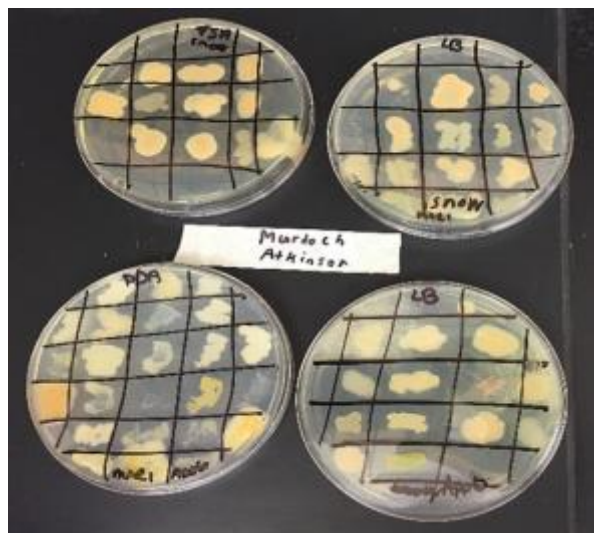


Figure 2. Master Plates for Storage of Subcultured Bacteria.

Of the 62 colonies tested, no zones of inhibition were detected, indicating that the bacterial isolates did not produce antibiotic compounds.

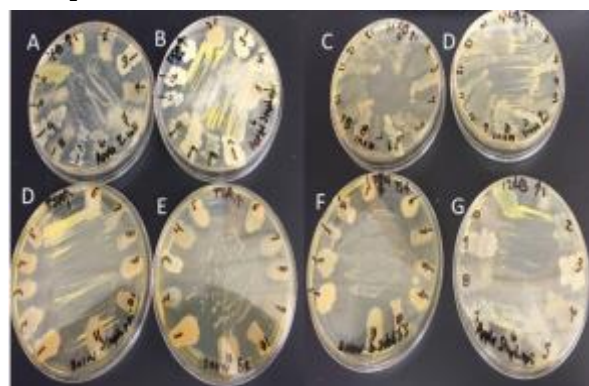


Figure 3. Bacterial Isolates Plated Against Safe ESKAPE Relatives to Test for Antibiotic Production. Bacterial isolates were plated against *E. coli* (A,C,E), *S. epidermis* (B,D,G) and *B. Subtilis* (F), using the patch/patch method.

Table 1. The Number of Colonial Isolates That Were Tested Against Safe Relatives of ESKAPE Pathogens for Antibiotic Production.

Soil Sample	Media	Total # of Colonial Isolates
Site 1	LB	13
	PDA	23
Site 2	LB	15
	TSA	11

Discussion

Purpose of Experiment

Research into antibiotic production is essential if we are to slow or halt the rapid increase of antibiotic resistance. While soil bacteria are the source of most antibiotics, the distribution of soil bacteria capable of producing antibiotics is not fully understood. Indeed, the ecology of soil bacteria itself is not fully known. This study aims to examine the possibility of finding antibiotic-producing bacteria in a site recovering from agricultural use.

Soil Collection

We chose to collect our soil samples from Church Farm because it has proven a valuable resource for research at Eastern Connecticut State University and has become a valuable nature preserve. The area was farm land since the late 1700s but has lain fallow

but has lain fallow for several decades, allowing the soil to regain nutrients and for native species to repopulate the area. The site is relatively unpolluted, surrounded by protected woodlands. This woodland system, combined with the Mount Hope River that flows near the property, supports a variety of plant and animal life. In the future, we will focus on nutrient-poor environments instead of habitats as rich as this since bacteria that are capable of antibiotic production may be more common in harsh environments where nutrients are in limited supply (Leisner et al 2016).

Cultures

The TSA, LB, and PDA plates with a dilution factor of 1:100, supported a high percentage of fungal colonies. We chose to avoid the fungal colonies when selecting colonies for subculturing to decrease the risk of

contamination and the risk of the fungi out-competing the growth of our bacterial colonies. Many fungi grow more quickly than bacteria, especially on PDA, which is commonly used to support fungal growth. In addition, fungi reproduce via spores, which can contaminate the entire petri dish. All plates were cultured at room temperature. For further experiments we may consider using the air temperature or the temperature of the soil from which the soil samples were taken to more closely match the natural conditions of the samples.

Antibiotic Production

From our 62 colonies tested, no zones of inhibition were detected, indicating that the bacterial isolates did not produce adequate amounts of antibiotic compounds to deter the growth of our tester strains of bacteria. This may be because our samples were taken from a nutrient-rich environment. It is possible that bacteria in such an environment do not need to produce antibiotic compounds to deter the growth of nearby bacteria as a means of competing for limited resources. Further literature searches indicate that the production of secondary metabolites with antibiotic properties is triggered in nutrient-poor environments (Rigali et al 2008). Various explanations have been suggested, and evidence exists to support many of them. These explanations include bacteria using antibiotic compounds in competition, signaling, and predation in response to decreased nutrient availability (Leisner et. al., 2016).

Conclusion

While no antibiotic-producing microbes were isolated during this study, we did gain valuable insight on increasing the chances of finding antibiotic-producing microbes. In the future, we will sample from stressed, nutrient-poor samples and will attempt to keep the temperature closer to that of the environment from which the samples were collected.

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