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A LABORATORY BASED STRATEGY FOR TEACHING MICROBIOLOGY TO AT-RISK SECONDARY STUDENTS

By

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ABSTRACT

A LABORATORY BASED STRATEGY FOR TEACHING MICROBIOLOGY TO AT-RISK SECONDARY STUDENTS

By

Joseph G. Dailey

The thesis documents the revision of the class, Tales From The Petri Dish, so that students will obtain a basic knowledge of what bacteria and fungi are and how this knowledge and microbiological techniques are used in the worlds of business and research. The primary means to accomplish this goal is the development of several meaningful laboratories and learning experiences including units on food microbiology and plant pathology.

The subjects in the study are students at an alternative high school which is a drop-out prevention program for at-risk students at the secondary level. The data collected includes pre-tests and post-tests, clinical interviews, descriptive demographic information and student evaluations of laboratory exercises.

Despite generally weak academic records prior to the class, all students achieved scores one to three times higher on the post-test as compared with the pre-test. Also, students in the clinical interviews reported positive attitudinal changes.

To Terri, for everything.

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INTRODUCTION

This document reports the revision of a class entitled Tales From The Petri Dish (PD). I taught two versions of the class, PD1 and PD2, prior to beginning my study. The first class was an attempt to put into practice some of the exciting lab exercises I did at Michigan State University's 1990 Cellular and Molecular Biology Workshop and the Frontiers of Physical and Biological Science Workshops held throughout the academic year. In the second offering, I organized the class around a central question after learning of this approach from Dr. Glenn Berkheimer at the Environmental and Behavioral Biology Workshop, also offered by M.S.U..

I teach at an alternative high school in southwestern Michigan. For reasons of confidentiality, it shall be referred to by a portion of it's acronym (AHS). The school opened in 1986 as a dropout prevention program of the sponsoring school district from which generally provides 60% of our student population. We accept students from six other districts in the county with whom we have reciprocating agreements. All of our students are at-risk for not completing high school and are referred to us for any number of reasons including but not limited to pregnancy, poor attendance, disruptive behavior, court referral or self-referral. Generally, AHS students are below grade level in most or all skills; some are well below. Class sizes are relatively small,

with 15 or fewer students. This is essential given the large amount of assistance and support that at-risk students require. Most of the student body is in the typical high school age range of 14 to 18 years of age. We also have a small but consistent percentage of adult students who are generally between 19 and 30 years of age. These adult students attend the same classes as the alternative students including Tales From The Petri Dish.

Another teacher (at one-third time) and I comprise the AHS science department, as a result course offerings are limited. We offer as basic courses, one year of biology and one year of physical science, generally taken by freshman and sophomores. Upper level classes one-semester long are Stream Team and Tales From The Petri Dish. Miscellaneous short courses with such names as Environmental Clean-up, Project Wild and Proficiency Test Preparation are also offered as needed.

AHS students select classes in consultation with the school counselor. I supply the counselor with a list of students that I think would be suited to the PD class; some of those students elect to take the class. The counselor generally follows my selections in fact, and always in spirit. There are scheduling constraints imposed by having a population of only 100 students and 7 to 8 instructors teaching a class during any one class period. Generally there will be a few students in each section that either have had the class before or were not on my list (because they are new students or PD is still the best available fit). I base my list on personal observations in and out of class using various subjective judgements such as maturity, intelligence,

perseverance, attitude and attendance. The students in the PD classes are some of the best at AHS. However, if these same students were at a traditional high school, it is my opinion, that they would for the most part rank in the lower half of their class.

Though difficult to quantify, students display many disruptive and self-defeating behaviors. One of those is poor class attendance. We have an 80% attendance policy: if a student is present less than 80% of the marking period (not including excused absences) they will not receive credit. In any given marking period (20-25 school days) anywhere between 5 and 20% of students lose credit. PD3 started with 12 students though only 5 attended enough classes to receive credit. The next section started with 12 students and 11 received credit. Our students are also fairly transient. Of the approximately 100 students who begin the year about half will be replaced by someone else by the end of the year.

Generally AHS is not a violent place. We have only a handful of actual fights each year and no-one has ever been apprehended with a gun, though pocket knives are fairly common. What is common is low self-esteem and it's results - apathy, disengagement and acting out.

In the 1995-96 school year, AHS serviced 168 students with approximately 100 of them being enrolled at any one time. Of these 168 students, 63 were referred to Time-Out (TO). These numbers do not include out-of-school suspensions. TO is a separate room and has two uses: in-house suspension and a place to send students when the instructor sees fit to

remove them from the classroom. In most cases a student is in TO for between 10 and 55 minutes, or one to three days in the case of an in-school suspension. TO is a boring place and most students truly prefer being back in the classroom. We have found negative aspects but it does allow one to carry on a classroom lesson without disruption. Additionally, students are not able to sit and enjoy the action in the office. Below is a breakdown of students by frequency of TO referrals.

Number of students with 15 or more TO referrals - 17

Number of students with 5 or more TO referrals - 23

Number of students with less than 5 TO referrals - 23

Number of students who had no TO referrals - 105

I use textbooks infrequently in my alternative high school classes. It is the informal policy of all AHS teachers to not assign homework. Consequently any reading for content takes place during class time and I prefer to use that time with students in more active pursuits.

PD was first offered in Winter Semester, 1993. The class had time available for relatively involved experiments because it met two and one-half block class periods per week over 18 weeks (a block class period is 110 minutes long). It's premise was to allow students to try a number of lab exercises on a wide variety of biological topics. Basically, my goals then and now are the same: help students to more meaningfully learn science, they should enjoy

the experience and they should be motivated to go further in science either through study or work. I believed that simply increasing the amount of "hands-on" learning I offered to students would accomplish these goals and to some extent that was true. Students did enjoy the opportunity to do more experiments. However, I do not think students learned a lot or were so engaged by the class that they would choose to pursue more science. I recognized that my students might perform better in a structured environment within which they are given responsibility. In contrast to that thought, PD1 was more of a biology experiment sampler without a strongly unifying theme. Also, Tales from the Petri Dish did not generate the same student excitement as my upper level environmental science class called Stream Team.

I attended the Environmental and Behavioral Science Workshop offered by M.S.U. at the Kellogg Biological Station in the summer of 1993. The culminating project of that summer was to produce a unit plan based on the formulation of a central question as explained by Dr. Glenn Berkheimer of M.S.U. I chose Stream Team to revise for my unit plan. In using the unit with students in the fall Stream Team class, I feel there was great improvement in student understanding though I have no data to substantiate this belief. Heartened by this academic improvement, I wanted to bring aspects of a "central question" to the PD2 class and chose as my theme "Microbiological Technology-How People Use Fungi and Bacteria." Some topics from PD1 and 2 are discussed later. I saw the results of these changes as

positive in terms of student achievement and reducing student frustration. Choosing to revise the Petri Dish class as my thesis project is simply continuing this trend.

I chose to continue teaching microbiology at AHS and therefore to use it as the area of my thesis work, for a number of reasons. I have had some upper level course work in that area. I have a personal interest in the subject and briefly considered pursuing a masters degree in plant pathology. From the students' point of view, microbiology has value in that it has a great impact on our lives, which will only increase as technology advances, in areas such as food production, health safety and medicine. For many AHS students, career possibilities literally do not go far beyond auto-mechanics and truck-driving. I think this is at least partly because of their backgrounds. They have few role models in other professions and many of their parents are unemployed. Given these facts, at-risk students are particularly in need of familiarity with and information about a broader range of career choices. I hope that PD will provide some of that for them.

Effective techniques for the instruction of at-risk students is not a topic that receives a large amount of attention in the science education literature. However, two common and inter-related themes emerge from what is available. One is that the course should be designed in a way that connects content with the real world. The second is that at-risk students have special needs in the area of engagement and self-esteem that must be addressed

before they can be effective learners. I believe these relate back to the three goals I mentioned earlier in the introduction. The need for varied pedagogical approaches to educate all students is also supported by the science education literature.

All students who receive science instruction do not learn equally well. Several studies have documented a wide range of outcomes. LaPointe (29) found that 14-year old science students in the US ranked 14th (of the 20 counties) in a survey of science and math achievement. The bottom 10 percentile of students had fewer than 35% correct responses. Clearly they have not learned science in a meaningful way.

Tobias (37) believes that successful programs for at-risk students should involve students with use of concrete materials in applying science principles. Hill and Hounshell (28) saw dramatic improvements in the test scores of high school students in a remedial summer biology class. The course was designed so that there was an emphasis on process skills and real world applications of information. Vatter (38) says that schoolwork for at-risk students should be "hands-on" and tied to real work in the real world.

Regarding the special needs of at-risk students, the context that has produced them must be considered (1,2). While these students have undeniable skill deficits, they too often possess the necessary intelligence but are simply not personally involved with their studies. For some reason they have learned to discount the value of schooling. Steele (36) discusses the experience of many African-Americans who have failed to thrive

academically. For African-Americans who have done well academically in one educational setting, the change to a different setting where they experience stigma has frequently led to academic decline. The stigma that Steele refers to is a lack of belief in an individual students' abilities based on their group rather than what the individual student has demonstrated. I am sure that this phenomenon of stigma is not restricted only to African-Americans and that other groups experience it on the basis of their class or another characteristic. Experience of this stigma is part of the context that produces at-risk students.

Newmann (32) refers to the opposite of this alienated behavior as engagement. Some of the characteristics of instruction that lead to engagement are that students feel membership in their school, that they are doing authentic work with a connection to the real world and that it has an element of fun. Yager (39), in discussing STS (Science, Technology and Society, a curricular focus promoted by the National Science Teachers Association), emphasizes that education should include a strong component of career awareness especially relating to science and technology.

In summary, my thesis problem is to revise Tales From The Petri Dish in such a way that students will obtain a basic understanding of bacteria and fungi and how this knowledge and microbiological techniques are used in the real world of business and research. The primary means to accomplish this goal are the development of several meaningful laboratories and learning experiences.

As mentioned previously PD 1 and 2 preceded my actual study but some valuable activities were developed. I shall briefly describe those that in a more highly developed form have been part of PD during the period of my study. The general pattern for lab experiences was to first discuss background information as a class. Then students would perform and report on the experiment. The report format is based on the Scientific Method as typically presented in most high school science textbooks. As a way to learn the reporting format students were asked to develop a question on any topic that seemed reasonable and practical. One example was "How consistently made are seemingly identical objects?" To answer this, the student individually weighed each one of a large number of paper clips on an electronic balance. Another student studied fly feeding preferences.

Students planned a one-day program of sampling for coliform bacteria at 20 sites on two local river systems using Redigel premixed selective medias. The intent was to develop a picture of where and to what extent fecal contamination existed on the PawPaw and Black River systems.

The first version of the experiment Bacteria Are Everywhere was in PD1. Students cultured soil and epiphytic bacteria on nutrient agar in order to observe the variety of the colony morphologies and "prove" that bacteria are present even though invisible to the naked eye. The students initially did not show a great deal of interest in what we had grown. Interest increased when I directed their observations by pointing out what I could see. In the course of discussion the statement was made "It's pretty hard to get excited about

something when you don't know what you're looking at". It had not occurred to me beforehand that students would want to identify the different types of bacteria we found so I wasn't prepared to help them do that. From this experience I saw two needs (which were addressed in the classes taught during the research period) - a better way to steer the students into closely observing colonies and something that would meet their need to know what they were looking at.

Using a tissue culture kit from Kemtec, we cultured carrot seedlings. I wanted to include tissue culture in the course because it relies on sterile technique and other microbiology skills and is a practical application of these skills. Seeds were placed on a germination media and remained there until seedlings had formed. Students transferred seedling stem sections to callus forming media and later transferred callus to cloning media. The resulting seedlings could then be potted. Contamination was present at every stage and resulted in only two seedlings being healthy enough to plant.

Obviously sterile technique was used in some of the activities from PD1 but in PD2 I presented it as a discrete topic. Students practiced sterile technique skills on their own and studied the newly developed handout entitled Microbiology Is... & Sterile Technique (appendix A). I found that later projects went more smoothly when students could concentrate on an experiment without the pressure of having to simultaneously learn sterile technique.

In the second version of the experiment Bacteria Are Everywhere we

grew bacteria from three sources - the air (dishes left open in various places for various lengths of time), infusions of distilled water and soil, field grass and beans, and the human body (coughing onto a plate and touching a plate with unwashed fingers). The bacteria which happened to grow were used in the next two experiments.

To direct students toward closer observation of colony morphologies than had occurred in PD1, I gave them a list of colony features. For a given colony they would make comments about each of these features. Each student would be responsible for reporting on several different colonies. This system did improve student observations and the time they were willing to spend on it. However, students did not have a broad enough vocabulary to express the differences that they saw. For example, a colony may have an internal filamentous appearance that can be one of six different patterns (i.e., arborescent). My students, in looking at that might say something like "it had a lot of hairs." When I would press them to be more specific they were generally unable to respond and experienced a high level of frustration.

In another improvement over PD1 regarding bacterial observation, students took samples of the cultures grown in class and made fixed slides using solutions from a Frey kit. We learned together as I had only done this once before in a college microbiology class. Due to poor technique, most slides were not viewable but students enjoyed the process of fixing and staining.

Using a Kemtec kit, "Enzymes in Industry", the class compared the

rates of juice production from applesauce treated with pectinase and untreated sauce. This experiment was relatively simple to perform but it did not capture student interest. I think this is because at-risk students are not aware of the importance of enzymes. Additionally, I had not provided enough information to correct this nor had I explained the importance of enzymes in other experiments we were doing in the class.

In keeping with the purpose of PD2 as stated in the syllabus, I took the class to the medical technology lab of a local hospital. Students viewed the equipment and techniques used in a modern facility and, where applicable, compared them with what we used in the classroom. I believe this allowed them to see an application for the skills and information they had been learning.

CHAPTER 1

SUMMARY OF SCIENCE CONCEPTS BEHIND EACH UNIT OF PETRI DISH

Microbiology is a very broad field of study and as a result it can be defined in different ways, depending on the perspective of the person providing the definition. My students might define it as "the study of organisms that are only visible under the microscope." I would have them add to this "and all of the impacts of the relationship between microbes and other organisms, especially humans." While my students might think that the role of microbiology is to look at such things as the physiology and reproduction of bacteria and fungi, my message for them is larger. For as long as humans have existed they have had a relationship to microbes. Microbes are responsible for diseases that have evolved along with us, not to mention their role in decomposition and the cycling of atmospheric gases. Humankind began in prehistoric times to harness some microbes in the production of foods and beverages. In the last few hundred years, through methodical scientific analysis, humans have begun to understand and manage microbes in the area of medicine, the environment, wastewater treatment, industrial production, plant pathology and others. In the

following section I have included the majority of the information I selected for my students to learn.

I. Sterile Technique (ST)

ST (34) is the most important basic concept for a laboratory based microbiology class for several reasons. There are many bacterial and fungal spores in our environment and that can have positive or negative results. It is good, for example, that decomposers are mobile enough during some portions of their life cycles to colonize new food sites and keep nutrients cycling through the environment. It is usually bad for the quality of foods in the home that these same decomposers are so mobile. As the concept of ST pertains to classroom activities, I stress that it allows us to study only the organisms of interest without contamination. The use of sterilizing liquids, such as alcohol and bleach, and different forms of heat for sterilization are discussed and practiced (autoclaving, flaming, boiling and baking). A petri dish is of most use when it is properly managed. In general, the student should be aware of the need to keep containers sterile and reducing exposure to contaminants when they are studying microbes.

II. Enzymes

Enzymes are proteins that serve as catalysts for chemical reactions. Originally discovered in yeast, it is now recognized that enzymes are found in all forms of life Because they are part of all life functions, enzymes are

essential for the existence of all organisms. Each enzyme performs a specific task and that specificity is a result of the unique shape possessed by each type of enzyme. Factors that affect the rate at which an enzyme functions include temperature, pH, presence of inhibitors and the concentrations of reactants. Extreme temperatures can denature an enzyme.

III. Bacteria

Bacteria are single celled prokaryotes and were probably the first form of life on the planet (33, 16). Some are photosynthetic but most absorb nutrient molecules through the cell wall and membrane. Bacteria most often reproduce asexually by simple cell division though they do have some characteristics, such as mutations, that introduce variation. The average doubling time is twenty minutes. At this rate one microscopic bacteria can produce a colony visible to the naked eye within twenty-four hours. Bacteria live in the widest variety of environments of any organisms on earth including aerobic and anaerobic conditions. Bacteria can be identified and classified in a number of different ways. The individual cells have one of three basic shapes - spheres, spirals and rods. Groups of bacteria ranging in number from a few to several thousand or more exhibit one of three grouping patterns - chains, sheets and clusters. Gram stains can be used to identify bacteria because the presence or absence of peptidoglycans in the cell walls of different types determines which of two stains are retained. Bacterial types vary upwards from zero in the amount of the enzyme catalase they

produce. Types of bacteria also vary in their level of resistance to commercial antibiotics. At the level of a whole colony, each bacterial type has visually observable, consistent physical characteristics when grown on solid media in a petri dish. Example of these characteristics include thing such as the colony's profile shape, the way the colony's edge is shaped, light transmission and so on. All of these characteristics can be used in the classification and identification of bacteria.

IV. Fungi

Fungi are an extremely varied group of organisms. In general they are multicellular eukaryotes where the cells are arranged in long strings called hyphae. Hyphae are not themselves reproductive structures; rather they are the phase of the fungal life cycle concerned with growth and acquiring nutrition. A mass of hyphae is called a mycelium. When the average person thinks of fungi they are usually picturing a reproductive structure like a mushroom or green bread mold. Of the total fungal biomass in the world most is in the form of these hyphae. Most fungi obtain food by externally digesting a food source and then absorbing the resulting molecules through the cell membrane. In addition to digestive chemicals, fungi are also capable of producing an incredible variety of compounds generally for the purpose of defense, an example being the antibiotic penicillin. Fungal cell walls are usually made of the polysaccharide chitin, a material also found in the exoskeletons of arthropods. Spores are produced by sexual and asexual means

and are thick walled in order to survive hostile conditions. Sexual reproduction occurs in most fungi, though the form of it varies widely.

V. Food Microbiology

The fermenting microbes are important in the production of many foods. Anaerobes obtain the energy to support cellular activities from their food sources through fermentation. Over the history of humankind we have selected microbes that produce desired compounds as by-products of normal fermentation. One example is the production of alcohol for beverages usually accomplished by one of a large variety of yeasts. Cheeses, butter, yogurt and sauerkraut are a result of lactic acid fermenting anaerobes. Carbonation, the "fizz" in beverages, is a result of the production of carbon dioxide by various microbes as is the rising of leavened bread.

VI. Plant Pathology

Two groups of organisms, fungi and bacteria, are responsible for the majority of diseases known to afflict plants (24). Of these, the fungi are responsible for most of the known diseases. In a typical successful fungal infection of a plant, a spore will land at an opportune exterior location. From there it will grow a series of structures designed to adhere to and enter the body of the plant. Hyphae or other structures will either enter inter-cellular spaces or the cells themselves and begin digesting and/or absorbing food. There are two major possible causes of an unsuccessful infection . One, the

environmental conditions are not appropriate for the pathogen; many fungi require humid conditions for spore germination. Two, the plant is not susceptible to that particular pathogen. There are several thousands of identified plant diseases but only 10 to 20 of them are able to infect any one species. Plant defenses are of two types, passive and active. The passive category includes solid barriers like the hard surface of a potato or chemicals that are always present such as the phenols that give pines their distinctive odor. Active defenses include the production and delivery on demand of special chemicals such as chitinase which breaks down fungal cell walls. Also, rapid local cell death (hypersensitive response) controlled by a host plant can prevent the spread of the fungus at the cost of only a few cells rather than the death of the entire plant. Plants and their pathogens are constantly evolving their defenses and offenses such that commercially developed resistances in food crops generally last no more than five to ten years. Since it's accidental importation in 1907, Chestnut Blight has decimated the American Chestnut (4,8,). The fungi responsible for the blight, *Endothia parasitica*, may be cultured in the lab.

VII. Tissue Culture

Tissue culture (5, 17,18) is the production of an entire organism originating from a small piece of that organism by the use of hormones, techniques borrowed from microbiology and specific culture conditions . Tissue culture is theoretically possible with complex animals though to date

only some animal cells and organs have been cultured. Meanwhile, perennial plants such as the hosta have been produced in commercially valuable quantities. In this process, plant hormones (such as kinetin and indole acetic acid) stimulate the formation of de-differentiated or callus tissue from a small piece of the original plant. Then large numbers of whole plants in seedling form sprout from the edges of the callus tissue. These may be removed, planted and grown in the same manner as any seedling. The growers at Walters Gardens have found that plant vigor increased in the first generation after culturing. Tissue culture differs from simple vegetative propagation in the need for sterile conditions, the formation of callus tissue and the larger numbers of plants produced from a single piece of plant.

CHAPTER 2

IMPLEMENTATION OF THE COURSE

I taught Tales From The Petri Dish three times during my research period: PD3 was Fall Semester 1994, PD4 was Winter Semester 1995, PD5 was Winter Semester 1996. A complete list of experiments and learning experiences is found later in this chapter. Briefly, the revised PD classes differ from PD1 & 2 in several ways. Some topics were dropped for various reasons and others added. The section presenting basic information about bacteria was restructured. A section about fungi was added. The study of the relationship between plant pathogenic microbes and plants, which included experiments to investigate Chestnut Blight and induced resistance in cucumber, was added. The selection of food microbiology laboratories changed - making cottage cheese was out, making sauerkraut, ginger ale, yogurt and brewing beer was in. Basic information about enzymes and additional experiments to demonstrate this information were added. Learning sterile technique was basically the same.

PD5 differs from it's two immediate predecessors in that I have dropped the topic of tissue culture. Because microbes are not involved in the

tissue culture process it is technically not microbiology, though it does use techniques derived from microbiology. My experience with tissue culture over the first four classes tells me that the problem of contamination makes the effort not feasible under conditions in my classroom. Students were enthusiastic at the start of the project, but the high frustration due to contamination and the fact that each step takes so long caused them to lose interest.

Some new teaching techniques were required to reach the goals mentioned in the introduction. In choosing which experiments to include I considered these goals by asking key questions. Would the experiment help students to more meaningfully learn the scientific concepts laid out in the previous chapter, Summary Of Science Concepts? Would my students be excited by and enjoy doing the experiment in terms of feeling and being successful? Would students doing this experiment possibly be motivated to explore the subject further? Other important factors were matters of practicality such as time constraints and availability of the necessary equipment.

The other reason for including these new teaching techniques is to make sure the students have an experience that reinforces each of the concepts in the post-test (see appendix J). To that end there is a field trip for each of the main topics of the course. I also wanted to fill out the work on bacteria and enzymes and provide work in the new areas of plant pathology, fungi and food microbiology.

Not all new teaching techniques were a new experiment or field trip. Discussed below are some changes in teaching style. As stated earlier, I opted to create a more highly structured environment than had existed in PD1 and 2. An experiment, preparation for an experiment or a field trip was scheduled for each day of the unit before the first day of class. By contrast, in PD1 and 2, I had left a great many choices about direction of the class up to the students. Students were responsible for making all the media used in class during the research period. In preparing for labs, I would often ask the students to design the procedures to be followed in order to achieve sterile conditions. Sometimes students did not finish time-sensitive experiments promptly or were careless so that their results were flawed. I could have easily taken responsibility and fixed the problem, but I think that my students need to see the consequences of their actions. This meant the students in these situations needed to repeat the entire lab. They were good humored about this and generally did not repeat the same mistake.

Since I was not using a textbook, there had to be one place where students could find explanations and summaries of the concepts they needed to know. I wrote and typed all the notes students would need (see appendices A through H) to meet this need. Each of the items on the post-test was included in the notes. I chose to use pre-written notes because I have found previously that the process of students taking notes while I lecture uses more time than should be necessary. Also, since my at-risk students just copy what I write on the overhead (in many cases words are mis-spelled and the

penmanship is unreadable even by the student) does the exercise in any way lead students to mentally process the information. Most importantly, the information is not retained for long by many of the students. Each time that I distribute pre-written notes, I make the bargain with my students that I will continue to present notes this way as long as they are willing to read aloud and maturely discuss them. The discussions have often been quite good and the pre-written notes are certainly no worse than the lecture/notetaking model.

I used a wide variety of sources to obtain the information presented in the PD classes. Most of the plant pathology information comes from personal conversations with and presentations given by Dr. Ray Hammerschmidt (25,26) and Dr. Dennis Fulbright (14,30). Both are professors at M.S.U. and have suggested experiments they thought suitable for high school students at their presentations. I had other useful conversations with Professor Tom Corner (9) and graduate student Michelle Anderson (3), also at M.S.U. All of the kits purchased from Kemtec (5,27) have arrived with well-written and in-depth manuals that allow me to adequately discuss the results with students while having more information to add if required. Another source was the lab write-ups distributed to participants of the 1990 Cellular and Molecular Biology Workshop and class lecture notes from the same. At a 1992 Frontiers of Biological Science session Fred Ham (19,20,21,22,23), a public high school science teacher, presented several labs which I have borrowed and adapted to my needs. Other books, journal articles and miscellaneous items I have used

can be found in the bibliography.

I. OUTLINE OF BASIC UNITS

PD3 & PD4 contained six basic units and PD5 contained five since tissue culture was dropped. A brief discussion of each is found below arranged chronologically with the approximate time allotted to each topic. A block class period is 110 minutes in length and one school week consists of five block periods. PD3 was taught between October 31, 1994 and January 20,1995. PD4 was taught between January 23, 1995 and March 20, 1995. PD5 was taught between January 22, 1996 and May 16, 1996. Since PD5 was taught over a longer period of time the reader should proportionately increase the amount of time shown below that was allotted to teach each unit.

Introduction — 1.5 weeks

At the start of the unit, a pre-test was administered to determine the beginning level of student knowledge of microbiology. Additionally in PD5, clinical student interviews (see appendix K) were conducted at the beginning and end of the class to determine student attitudes regarding microbiology and their knowledge of the subject. Analysis of that information may be found in chapter 4. The majority of time, however, was spent providing students with information about enzymes and basic skills necessary to perform later labs.

All aspects of sterile technique needed for successful completion of

later lab experiences are introduced at this point. The skills were refined and practiced in the lab experiences.

Understanding the nature and functions of enzymes is important to an understanding of microbiology since they are one of the primary ways that microbes act on their environment (i.e., how microbes obtain nutrients). In large part, food microbiology and plant pathology are examples of disciplines that study the action of microbes in the environment. For almost all students that have entered my classroom, enzyme is a meaningless word. If I want them to understand what a microbe is doing, I must teach them what an enzyme is.

Tissue Culture (PD 3 and 4) 0.5 weeks

I saw the tissue culture facility at Walters Gardens on a tour in 1981. I found the concept very exciting. As stated earlier, I included tissue culture in the course because it relies on sterile technique and other microbiology skills and is a practical application of these skills. Technically, the culturing process was started just after the pre-test since the entire process takes every bit of nine weeks. Also, it is still an unusual technique to use in a high school classroom and I wanted the students to experience something unique.

General Information about Bacteria 2 weeks

This unit contains critical information for appreciating these microbes and understanding later topics, especially food microbiology. I did not treat this as

a distinct unit in PD1 & 2 nor did I cover as much material. It became apparent that students entering my classroom knew very little about bacteria other than myths and misunderstandings. Therefore, in order to increase understanding and reduce frustration, more classtime was spent on covering basic information about bacteria. Also, since I already had most of the required equipment, performing the appropriate experiments was very feasible in my classroom.

General Information about Fungi 1 week

The same comments made for the General Information about Bacteria unit can be made about this unit also.

Food Microbiology 2 weeks

This unit has been a prominent part of the class since PD2. Since everyone eats food, microbiology fits into all students' prior knowledge. I assumed that it would be the best applied topic for raising student interest. Additionally, students would be able to eat and drink the products and use what they learn at home immediately.

Plant Pathology 2 weeks

Fungi are the primary disease agents of plants so this unit fits well into microbiology. Plant pathology is a "hands-on" way to look at microbiology in disease situations without the risk of infection that would come with

studying human pathogens. As with tissue culture, plant pathology offers an opportunity for an experience that is unique in the high school classroom. In addition, I have a personal interest in the topic and have often found that I do my best teaching when that is the case.

Conclusion

A post-test, which is included in the appendix, was administered at or near the end of the term scheduled for the PD classes. In PD5 clinical entrance and exit interviews were conducted with the same group of four students. A third evaluation tool administered in the PD5 class was a student evaluation of the experiments and field trips in a self-report format.

II. LIST OF FIELD TRIPS AND LAB EXERCISES

Following is a listing of each lab exercise and field trip that students experienced in PD 3 through 5. Summaries of each activity are found following the list. Activities on the list marked with an asterisk are either new since PD1 and 2 or significantly altered from the form they had in those early classes. Some exercises did not occur in all three classes during the research period. These exercises are labeled on the list with parentheses enclosing the numbers of the classes in which they did occur. If no class number is shown the exercise occurred in all three classes.

FIELD TRIPS
Walters Gardens* - Tissue Culture (PD3 & 4)
Campbell's Fresh* - General Information About Fungi
Hospital Medical Testing Lab - General Information About Bacteria
St. Julian Winery* - Food Microbiology
Collecting Chestnut Bark* - Plant Pathology

LAB EXERCISES
Tissue Culture (PD3 & 4)
Enzyme Characteristics
- Degradation of Lactose*
- Silver Retrieval from Photographic Film
- Juice Extraction from Apple Sauce
- Gelatin and the Effect of Boiling on Enzymes in Pineapple*

Sterile Technique
- Making Culture Ready Petri Dishes including Potato Dextrose Agar *
- Use of NiChrome Transfer Loops and Miscellaneous Skills

General Information about Bacteria
- Bacteria R Everywhere*
- Serial Dilution to Determine Food Contamination (simulation, PD4)*
- Using Selective Media to determine aquatic fecal contamination
- Bacteria Identification -
  - Making Stained Permanent Slides
  - Colony Morphology*
  - Catalase Reaction* (PD4 & 5)
- Isolating Pure Cultures (from the Bacteria R Everywhere plates)
- Looking at Bacteria with Microscopes*

General Information About Fungi
- Culturing Fungi in classroom* (PD4)
- Fungus Garden* (PD3 & 5)
- Spore Print (demonstration)*
- Serial Dilution to find best concentration of molasses and yeast (PD5)

Food Microbiology
- Brewing Beer*
- Making Sauerkraut*
- Making Ginger Ale*
- Making Yogurt*

Plant Pathology
- Culturing and Transferring Bark Carrying Chestnut Blight*
- Assessing Virulence of Chestnut Blight Strains (PD3)*
- Staining for Lignin* (PD4)
- Acquired Systemic Resistance in Cucumbers*

Microbiology Meal* (PD4)

III. AUDIO-VISUAL AIDS AND OTHER DEMONSTRATIONS

A. PD calender - Self-developed

At the start of each semester since beginning my research I distributed a PD calendar to the students. The calender is in a Monday-through-Friday format and creates for the student a "map" of the class. Listed are the approximate dates for covering the five units of the class, when we will do each experiment, important tasks, and, when applicable, times and destinations of field trips. Also listed are dates of the pre-test and post-test, and any early-release or no-school dates.

B. TC slides - from Kemtec Tissue Culture Kit

A set of six photographic slides comes with the Kemtec Tissue Culture Kit. They show what the plant material should look like at each stage in the tissue culture process. This is useful because students may look at the slides to compare classroom results with the desired results rather than relying on my knowledge. In this way they are required to take responsibility for making judgements for such things as knowing when to transfer tissue to a new plate.

C. Fungus/Plant Pathology Photographic Slides - from Dr. Hammerschmidt

This series of twenty-six slides is used as part of my introductions to both the Fungi and Plant Pathology units. I emphasize different aspects of the images in each of the introductions. Most of the slides show disease agents and/or the symptoms they produce in their host plants. The images are

useful because they make the variety and effect of plant diseases more real than any other method I have available in the classroom. Interest is generally high. I believe this is because we have an active two-way discussion of what is viewed. Also, perhaps, being at-risk and adolescents (for the most part) they enjoy the images of "gross" things. The slides were obtained by copying those that Dr. Ray Hammerschmidt uses in an introductory university course. The notes which accompany presentation of the slides are in appendix G.

D. American Chestnut Dried Specimens - Self-developed

Since the American Chestnut is rare, students are generally unfamiliar with it and need to be educated about it's gross morphological characteristics. I have collected chestnut branches and leaves, seed casings, dried flowers, cankered bark and nuts from various locations to show to students. Looking at these props is useful, especially when we go to collect cankered bark, because the students have a better idea as to what they are looking for. Also, given the lack of botany experiences in most K-12 systems, I believe there are intangible benefits in students seeing all of these plant parts together.

E. "Cultural Characteristics of Bacteria" - from Dr. Tom Corner

I acquired this four page key to bacterial colony morphology from Prof. Tom Corner of the M.S.U. Microbiology Department after PD2. It has several diagrams to illustrate descriptive terms that students can use to classify a

colony according to it's profile, edge texture, transparency, overall shape and internal texture. I ask my students to observe bacterial colonies on petri dishes so that they can develop an appreciation for their variety and because observation is indispensable tool of science. Students can readily access a broad vocabulary by matching the live colony to the pictures and then simply writing down the appropriate descriptor. Students in PD1 and PD2 attempting to describe the appearance of colonies without the key experienced a great deal of frustration because my expectations were greater than their verbal skills and vocabulary could meet. Also, importantly for at-risk students, the hand-out provides structure since students know what they are looking for, reducing fear of the unknown.

F. Prepared Microscope Slides - from Carolina Biological Supply Company

These prepared microscope slides show the three most common bacterial shapes (bacilli, spirilli and sphere) and typical ways that bacterium will group together (sheet, cluster and chain). After observing these closely, students are able to identify the shapes and grouping patterns on fixed slides that they prepare from live cultures more than half the time. Students also look at prepared slides of fungi for such things as reproductive structures.

CHAPTER 3

SUMMARIES OF LABS AND FIELD TRIPS

Students evaluated most of the labs and field trips in a self-report format at the end of PD4 and 5. The three numbers found below many of the following summaries are results from the student evaluations. For each experience listed on the evaluation students were asked to assign a range of values from 1 to 5 as answers to three questions. Question one was "How much did the experiment grab your interest?" Question two was "Do you think you understood the experiment?" Question three was "How much did the experiment help your understanding of the topics in the course?" The scores were derived by finding the percentage of positive response to the question. The more frequently that students answered a question positively, the closer a score is to 1.0 The ratings can be interpreted much like a grading scale; values of 0.90 and above are very good and a value between 0.60 and 0.70 indicates the experiment was acceptable but could use improvement.

I encouraged students to be honest, not to worry about my feelings and that nothing they said would affect their grades. Students appeared to take the opportunity seriously and gave a variety of values as answers in PD5. In

PD4 only six students completed the evaluation and usually four or five of them gave the same high value to all the activities. It would seem to me that students who write nearly all of one value simply want to be done with the task quickly. In PD5 all 15 students completed the evaluation and the values appear to be significantly less clustered. For these reasons I consider the evaluations by PD5 students to be valid evaluative information and these are the only values shown.

I. FIELD TRIPS

All field trips were taken during or near the end of the unit of which they are a part. I chose these points, rather than using the trips as part of an introduction, because my previous experience indicates that at-risk adolescents need prior familiarity in order to understand and develop an active interest in what they are seeing. For each field trip students were to return a report form to me. The questions were similar on each form. A sample, from the St. Julians Winery trip, is included in appendix L.

A. Walters Gardens Tour- PD4 only

We toured Walters Gardens as part of the tissue culture unit. Walters Gardens is a producer and wholesaler of perennials specializing in hostas. Walters begins the tour with a slide show describing the business as a whole and the particular methods of tissue culture used at the facility. Unfortunately, the bulk of the walking tour does not focus on tissue culture

but on the complex of warehouses, cold rooms, packing rooms, fields, greenhouses, etc. Approximately ten minutes were spent in the tissue culture rooms which is comprised of a media preparation room, an incubation room and plant processing room, the last two being sealed and sterile. Students were generally enthusiastic about tissue culture at the start of the unit but between the experimental failures discussed later and the nature of the tour that enthusiasm quickly declined. I feel this was because of the seemingly extraneous walking through warehouses and the like and that students were kept from getting close to the activity in the tissue culturing rooms.

B. Campbell's Fresh Tour

This is probably the best all-around of the trips for combining student interest and seeing the application of microbiology. Students are able to touch most everything and get up close to see. Our guide all three times was friendly, humorous and full of information. This is a very large production facility so it was possible to see all stages in mushoom culture. They are:

1) Composting of manure (to which water, gypsum and cottonseed meal are added) for one month in a large open air shed.

2) After the micro-organisms in the compost have done their work the majority of them must be killed. This is done in large rooms where the compost is steamed at 65% moisture between 150 and 180°F for several days. By this point the compost has been loaded into mobile 8' X 8' X 1' beds.

3) Mycelium of the desired species comes to the facility in bags that are kept

refrigerated. Campbell's has a lab in Kentucky where they grow the mycelium under optimal conditions with an eye towards quality control. Students can handle these bags and make the connection between those white threads inside and specimens we have seen back in the classroom.

4) Mycelium and the prepared compost are mixed together and placed in the spawn rooms where, over the next two weeks, the mycelia spread throughout the beds.

5) To stimulate formation of mushrooms the environmental conditions are altered by adding a layer of peat moss, raising the humidity, lowering the temperature and increasing the level of CO_2.

6) Mushrooms are harvested by hand over 3 to 4 "breaks" or crops. The mushrooms are then packaged and shipped. Of course the thing that widens the student's eyes is that the mushrooms have not been washed prior to being sent to stores. They both love and are repelled by that.

Interest-0.98/Comprehension-.96/General Understanding-0.96

C. St. Julian Winery Tour

This is the most anticipated tour due to alcohol's allure for teenagers. As a local tourist attraction, St. Julian offers tours every half hour followed by a tasting session. Groups are run through the plant rather quickly and as a result there is little time to pursue things of special interest to my students as we encounter them. Students see several aspects of the operation: grape processing (where they are crushed and screened); aging (where special oak

barrels and fermentation locks are of interest); viewing the high pressure metal champagne vats and the bottling room. In PD5, I mentioned to the guide that we were a microbiology class and she arranged for us to visit the St. Julian lab. The purpose of the lab is quality control and they monitor such things as alcohol content, color and suspended solids The value of this field trip is uneven, the ability of the guide to answer questions seemed to vary greatly and was affected by whether or not tourists accompany the group. But there is fun to be had by students standing around the sampling bar and ordering drinks, albeit non-alcoholic ones. See report form in appendix L. Interest-0.95/Comprehension-.87/General Understanding-0.89

D. Collecting Chestnut Bark

After much searching and invaluable assistance from M.S.U.'s Dr. Fulbright, I located a hill five miles from school with about a hundred clumps of American Chestnuts. Considering the efficiency of *Endothia parasitica*, the fungal blight that has ravaged these trees, this is quite a stroke of luck. In addition, the owners have generously agreed to allow me to bring classes there occasionally to see the trees and take some bark samples for culturing in the classroom. Students may never again see this many American Chestnuts and they can also clearly see the effect of the Blight on the trees. Students look for the cankers caused by *E. parasitica* on the dormant and leafless trees and cut out a small section of bark containing the characteristic orange-red pustules. Students see how the clumps of American

Chestnuts sprout from the stumps of long dead larger trees and see for themselves the woods' rot-resistance in the form of large standing snags. I feel this is a valid opportunity for my students to see that microbiology can include outdoor field work which appeals to some persons more than a career spent predominantly indoors. The last stop is an isolated, live American Chestnut that is approximately 150 feet tall and whose circumference can be enclosed by 4 people holding hands with arms fully extended. This trees' beauty and tenacious grasp on life despite being infected by the blight is impressive.

Interest-0.85/Comprehension-.89/General Understanding-0.91

E. South Haven Hospital Medical Technology Lab (PD5)

As in PD2, I took the class to the medical technology lab of a local hospital. Students viewed the equipment and techniques used in a modern facility including a blood cell counter, blood chemistry analyzers and a microbiology bay. I believe this allowed them to see an application for the skills and information they had been learning. As the microbiologist was explaining how she might identify various pathogens I overheard students commenting on how similar many of the pieces of equipment were to those at school and how the microbiologist's duties were similar to what we had done in class.

Interest-0.83/Comprehension-.85/General Understanding-0.83

II. LABS

A. Tissue Culture

There were two differences between methods of tissue culture attempted by the PD3 and 4 classes (5,17,18). The techniques described in appendix M were used in PD3 but not in PD4. One was the method for germinating the seedlings. The second was the type and sequence of media used in the rest of the tissue culturing process. If contamination had not become a problem the Murashige & Skoog medias we used in PD3 (A, B, C and Control) would have been useful for demonstrating the effect of varied hormone concentration on callus formation.

In PD4 I tried a kit from Kemtec using radish seeds instead of the carrot seeds provided in the kit. I had previously used this same kit and was able to plant a few carrot seedlings produced by the tissue culture process. Due to miscommunication between myself and the students, the callus forming dishes were sealed with Parafilm instead just being left in a closed, sterile plastic bag as recommended in the kit instructions. Probably as a result of insufficient gas exchange the seedlings didn't form callus very readily. When this mistake was discovered we removed the Parafilm and left the dishes in a plastic bag as recommended. Contamination was again a major problem. Since it was late in the marking period by the point that the problems were recognized there would not have been sufficient time to complete the process so the next step, transferring the stem sections to cloning media did not occur.

B. Enzyme Characteristics

The first two labs are taken from Fred Ham, a high school teacher, who presented them at a Frontiers Workshop. The second two labs come from a Kemtec kit entitled Enzymes in Industry.

1. Degradation of Lactose

Individual students have two containers of milk (21). One is untreated and the second is treated by adding Lactaid, containing lactase which degrades lactose into galactose and glucose. The containers are allowed to sit in a refrigerator overnight. On day 2 students taste milk from the two containers to see if they can discern a difference in sweetness. In addition, the students dip urinalysis glucose strips into the two liquids and compare this with the accompanying color chart. The color chart results correspond to values on Masterson's relative sweetness scale. Lactaid drops or crushed pills work equally well .

Interest-0.62/Comprehension-.84/General Understanding-0.89

2. Gelatin and the Effect of Boiling on Enzymes in Pineapple

Gelatin is freshly prepared but before it is allowed to harden, four different liquids are added: juice from canned pineapple, juice from fresh pineapple, and solutions of the commercial tenderizers Adolph's and McCormick's (22). The different gelatins are refrigerated overnight and observed the next day. Typically the gelatin treated with canned pineapple juice is the only one that

will set up firmly. Students are asked to hypothesize about why that happened. Obviously the pineapple's enzymes have been denatured by heat in the canning process.

Interest-0.71/Comprehension-.84/General Understanding-0.86

3. Silver Retrieval from Photographic Film

The students put small strips of unhardened, exposed photographic film in test tubes and add an aqueous solution of protease (27). The film strips are a "dispersion of silver halide in gelatin on a firm backing." The protease enzyme has the ability to degrade the gelatin as it is a protein. Within 20 minutes the dark emulsion which contains gelatin has obviously started to slough off some of the strips due to protease action. The literature that comes with the Kemtec kit informs us that a portion of the dark precipitate is silver.

Interest-0.78/Comprehension-.77/General Understanding-0.78

4. Juice Extraction from Apple Sauce

The students set up two funnels and graduated cylinders each containing filter paper and identical amounts of apple sauce (27). One batch of sauce has been treated with the enzyme pectinase, the other is untreated. As pectinase breaks up the pectin, water remaining in the plant tissues is released from the treated apple sauce, which consistently produces a greater volume of juice.

Interest-0.72/Comprehension-.80/General Understanding-0.88

C. Sterile Technique

1. Miscellaneous Skills

Except in one case, all media used in the class were made by the students (34). Each section produced approximately 120 nutrient and potato-dextrose agar plates. During the preparation of the first set of agar plates several other new skills and methods were acquired by the students. These include sterilization of work areas with a bleach solution, uses of Parafilm, inverting dishes with and without inoculated media to avoid condensation landing on the surface and flaming flask mouths. See appendix A for details.

Interest-0.69/Comprehension-.76/General Understanding-0.79

2. Use of NiChrome Transfer Loops

Students practiced effective use of transfer loops, using plain water and empty non-sterile plates. When I was satisfied that they had attained this skill students were allowed to proceed to the next unit of the class including the Bacteria R Everywhere experiment.

Interest-0.75/Comprehension-.87/General Understanding-0.87

D. General Information about Bacteria

1. Bacteria R Everywhere

As a whole class, students brainstorm possible locations where bacteria might be living. We prioritize the list of locations to insure variety and then students choose one or a few locations to investigate using previously learned

microbiological techniques. Students are required to write an experimental proposal detailing their hypothesis or question, what will serve as the experimental control, number of replicates, how experimental conditions are controlled, data recording format and procedure. Once I am satisfied with their proposal, students may perform their experiments and then report their results. Reports are graded based on the students handling and interpretation of the data.

Interest-0.84/Comprehension-.88/General Understanding-0.88

2. Bacteria Identification

a. Making Stained Permanent Slides

After learning by study of commercial prepared slides the shapes of individual bacteria and the three grouping patterns (chain, cluster and sheet), students make there own slides (13). They find a culture of interest from their Bacteria R Everywhere plates, fix them on a slide and then stain with one of six stains (saffranin, crystal violet, etc). The students then must microscopically determine the shape of a bacterium and it's grouping pattern.

Interest-0.90/Comprehension-.91/General Understanding-0.91

b. Colony Morphology

Using a diagram (9) that illustrates descriptive terms, students classify a colony as to it's color, profile, edge texture, transparency overall shape and internal texture. This works very well because it supplies structure in the form of pictures and vocabulary words to match up with the appearance of

colonies. I overlooked colony morphology and the catalase reaction when writing the form for the student evaluation of labs. However, based on their comments I would estimate that students liked the latter more than the former and that both would have had values in the range between making stained permanent slides and isolating pure cultures.

c. Catalase Reaction

Bacterial species vary in the amount of the enzyme catalase that they contain (3). This can be demonstrated by putting drops of hydrogen peroxide on part of a colony. I had my aide find 3 or 4 of the most widely differing cultures and we used those as a reference for a scale of reactivity. The students determined the reactivity of their cultures and added this to information they compiled regarding characteristics of bacterial cultures.

3. Isolating Pure Cultures

Again, using the Bacteria R Everywhere plates, students transferred and streaked cultures of interest onto new plates in order to determine if those cultures were pure. The students as a group were not highly engaged by doing this. It did work well however to assign the majority to an un-supervised task while I worked with two students in choosing and trans-ferring the cultures. These students enjoyed the experience.

Interest-0.67/Comprehension-.71/General Understanding-0.75

4. Using Selective Media

Students obtain water samples from a creek using sterile technique to determine the level of aquatic fecal contamination (31). The samples are then used with EPA approved materials from RediGel in a two step process. In the first, the petri dish media additive Violet Red Bile (VRB) encourages the growth of coliform bacteria and discourages the growth of any other bacteria. These colonies are counted and then results are expressed as number of colonies per 100 ml. A number of these colonies are transferred using sterile technique, to a second dish containing the additive Eosin Methylene Blue (EMB) which shows by a color change if any of them are the non-pathogenic E. coli bacteria usually associated with the feces of mammals. This media also discourages the growth of other bacteria.

Interest-0.63/Comprehension-0.70/General Understanding-0.73

5. Serial Dilutions

The technique of the serial dilution is useful in determining the concentration most appropriate to a particular situation. The PD4 students applied this technique to determine food contamination in a simulation (6). After a brief discussion of the math involved and food contamination measurement methods, students made tenfold dilutions (1:1 to 1:10,000) of colored water observing the decreasing color. They reported to me on the value of such a technique in the food industry and procedures involved.

In PD5 serial dilutions were used to "determine the optimal

proportions of yeast solution and molasses-water mixture needed to produce the greatest amount of carbon dioxide gas" (11). I gave some background information but it was the student's responsibility to find a way to determine the optimal proportion. Generally individual students tried only one or two different proportions and the group realized the next day that because of this they were not close to an answer. I explained how having a range of several different proportions from very little yeast and lots of molasses to the other extreme would cover all the possible proportions. The students were then able to adequately determine the optimal combination of yeast, molasses and water on their next attempt.

Interest-0.78/Comprehension-.78/General Understanding-0.80

E. General Information About Fungi

1. Culture fungi in classroom

In PD3 and 5 we constructed a Fungus Garden (10). An aquarium was prepared with a moistened 1" sand layer. Students placed objects (foods, fabric, etc) in the aquarium and then sealed it. Over time a variety of fungi grew and the Garden came to be "gross", in the students' words, which made it attractive to adolescents. The students were enthusiastic about setting up the Garden but remembering to make informal follow-up observations required prompting.

In PD4 we attempted to culture fungi in petri dishes (12) but it was less

entertaining which is why I returned to the Fungus Garden for PD5.

Interest-0.87/Comprehension-.90/General Understanding-0.88

2. Spore print (demonstration)

Using large mushroom caps from Campbell's Fresh, the students and I made spore prints. This is done by leaving a fresh, de-stemmed, mushroom cap undisturbed on top of a piece of paper. We fixed them onto the paper satisfactorily using hair spray.

F. Food Microbiology

The first two food microbiology projects below depend on fungi, the second two on bacteria. Brewing is the most complex process; the other three can basically be set up by students within 15 to 30 minutes. One problem is that with the exception of beer, these foods are not normally eaten by my students. As a result the enthusiasm was not as strong as I expected. I may substitute other foods in future classes.

1. Brewing beer

When brewing in the usual manner, it is not desirable to open and close the container each day due to the chance of contamination. I wanted students to trace the changes in the yeast population (7) and amount of alcohol in the beer on a daily (or nearly so) basis. In order to do this we mixed one master batch, poured it into the bottles and allowed brewing to proceed in vitro (see

appendix N). We saw a large jump in the yeast population and alcohol content during the first three days. Considering the conditions under which we made it I was not interested in drinking any of the beverage, however, the color, odor and head produced seemed very similar to commercial products. Students, as anticipated, were very enthusiastic about this project and did not fail to follow-up.

Interest-0.96/Comprehension-.89/General Understanding-0.89

2. Making Ginger Ale

Students mix grated fresh ginger root, sugar and other flavorings with yeast so that the whole will ferment (20). After the mixture is allowed to sit at room temperature for at least six hours, the solids are filtered out which stops fermentation and yields a moderately carbonated beverage. A difficulty is in the timing because the fermentation would not be completed until after the end of the school day. I asked students remember to come back in order to refrigerate the mixture overnight and get it out of the refrigerator at the start of the school day so that fermentation is completed. The students have remembered to complete these tasks about half the time.

Interest-0.87/Comprehension-.85/General Understanding-0.85

3. Making Sauerkraut

Students finely chop green cabbage, add 2.5% salt by weight and mix (23). The mixture is put into a container and allowed to sit at room temperature for a

period of a few weeks. Lactobacilli naturally found on the cabbage proliferate in this environment and through the production of proprionic acid (that characteristic sauerkraut smell) transform the cabbage into kraut. Finding some way to continually compress the kraut improves the process. The salt pulls juice out from the cabbage so there is a liquid environment for the bacteria. As the lactobacilli continue degrading the cabbage there is a decline in volume of approximately 25%. One quart jar out of the 12 produced in PD3, 4 and 5 was eaten. Our school librarian reports that it tasted acceptable.
Interest-0.68/Comprehension-.85/General Understanding-0.83

4. Making Yogurt

The process of making yogurt is quite simple (19). Microbes in the raw material, skim milk, are killed by heating. After the milk mixture cools and acidophilus bacteria are stirred in, it goes into sterile receiving containers which are closed and incubated at room temperature. Yogurt should form in one to two days. Students in PD4, at first hesitantly and then readily, consumed their yogurt at the Microbiology Meal (discussed later). Students in PD3 and 5 did not eat their yogurts because, I suspect, I did not set aside special time for tasting it.
Interest-0.70/Comprehension-.84/General Understanding-0.84

G. Plant Pathology

This section is the most problematic, most original and my favorite. Drs. Fulbright and Hammerschmidt had not written up these experiments for use in the classroom or tested them with high school students. Consequently they are still in early stages of development and adaptation to my classroom. Also, proper scheduling of events in these lengthy procedures, (lengthy at least by the standards of at-risk secondary students) has proven so far to be difficult.

1. Culturing and Transferring Bark Carrying Chestnut Blight

After collecting samples of bark (see field trips) it is cut into dime or quarter sized pieces. The exterior is "sterilized" by soaking in dilute bleach and then the piece is pressed lightly onto a potato dextrose agar plate (see appendix O). Within 7 days those pieces that will produce a blight mycelium will have done so. Blight fungi cultured in this way may be assessed to determine it's virulence (see next section). One significant problem has been contamination of the plates by an unidentified green fungus. Dr. Fulbright suggested trying different bleach concentrations and soaking times. In PD5 we had much better success when soaking the bark pieces in 30% and 40% bleach solutions as compared with the 10% bleach solution Dr. Fulbright had originally suggested.

Interest-0.85/Comprehension-0.83/General Understanding-0.85

2. Assessing Virulence of Chestnut Blight Strains

It is possible to determine virulence by looking at a culture's growth form on a petri dish but a more definitive test is available. Students remove a small plug from a Golden Delicious apple using a hand cork borer (see appendix O). They use the same size bore to get a plug of the mycelia and place that in the apple. Within 14 to 21 days an adequate portion of the apple will have rotted to determine virulence. If the rotted area has the correct size and shape it is virulent. If contamination has occurred the rotted area will be a color other than medium-dark brown.

Because of contamination and scheduling problems, PD3 was the only class to do this experiment so no values from the student evaluations are listed. I would estimate the interest and enthusiasm of that group for this experiment to be in the high moderate range. I was not entirely certain in PD3 that the mycelia we chose to test for virulence was Chestnut Blight because the results we obtained were inconclusive. Much of what is written above comes from conversations with Dr. Fulbright subsequent to and regarding the experience in PD3. Obviously this experiment needs refinement but I believe further efforts are worth-while given it's score for question 1 (interest, rank of fourth overall) given to the lead-in experiment, Culturing and Transferring Bark Carrying Chestnut Blight.

3. Staining for Lignin

One defense mechanism that plants employ after being attacked by pathogens is the creation of tough, lignin bearing walls. In order to see this students remove the outside layer from cucumber seedlings and stain them for lignin using 1% phloro-glucinol and HCl (25). Typically not much is seen at this point. Then the seedlings are inoculated with spores of the pathogen, *Colletotrichum lagenarium*. The inoculated plants are kept in damp, dark location for two to three days before the students again peel and stain the outside layer. This time some areas of lignin are plainly visible and on close inspection even the fungal structures stimulating this phenomenon can be seen. Students commented that they would have liked to spend more time on this experiment.

4. Acquired Systemic Resistance In Cucumbers

Ray Hammerschmidt declared in his Frontier's presentation that plants have something like an immune response. That seemed like a novel and intriguing way to look at things. He also maintained that it would be possible to demonstrate this in the classroom, which is the point of this experiment (see appendix P). The premise is that if a cucumber is inoculated with a non-killer pathogen then it will be stimulated to resist a second inoculation by a killer pathogen. My classes were unable to actually complete the experiment. In PD3, I had not allowed enough time for the cucumber seedlings to grow given the low light conditions in December and January. In PD4 and 5, the

plants were ready. However, we were unable to produce viable cultures for the inoculations. Although the experiment was not completed, students experienced a reasonable approximation of the experiment because of three things. They read and intelligently discussed the hand-out I developed. The students raised the plants to the extent possible. They also saw a photographic slide comparing the effect of *Colletotrichum lagenarium* on two cucumber leaves. One leaf was from a resistant plant and the other leaf was from a susceptible plant. It is clearly visible in the picture that the resistant plant's leaf was inoculated in roughly the same pattern as the susceptible plant's leaf except that the former exhibits none of the necrotic lesions found on the latter. Additionally, 60% of the PD5 students correctly answered the question about this test on the post-test. Given these facts I conclude the experiment is within the abilities of at-risk students and will continue to use and perfect this experiment in future PD classes. Interest-0.81/Comprehension-0.77/General Understanding-0.77

H. Microbiology Meal - This idea came from the students and a how-to article by Gillen and Williams (15). Students in PD4 expressed an interest in a party at course end. I countered by suggesting a Microbiology Meal. The concept of the Microbiology Meal is that all food and beverage items consumed, except for condiments and flavorings, must have somehow been produced by microbes . "Produced" can be widely interpreted if need be. The students must be able to verbalize the microbial connection. On the day we planned

the menu the students as a group suggested the foods and gave their microbial credentials. Our menu was this:

mushrooms (from our tour of Campbell's Fresh)

hamburger (serial dilution simulation of measuring food contamination)

yogurt (produced in class)

ginger ale (produced in class)

potatoes (-dextrose agar and the Black Scurf sclerotia on the skin)

cheese

milk (can be made into yogurt)

This activity only occurred in PD4 and I did not ask the students about it on the lab evaluation form though I am certain they would have given it high values. There was no Microbiology Meal in PD5 for reasons having nothing to do with it's value as a learning reinforcement activity.

CHAPTER 4

EVALUATION OF THE COURSE

I. Pre-test and Post-test Statistics

The numerical results of the pre-tests and post-tests for each student in PD 3, 4 and 5 are listed in appendix Q. A complete version of the post-test from PD5 is in appendix J. The pre-tests and post-tests previous to that, while not identical, are virtually the same. Obviously any questions about tissue culture were not included in the PD5 final exam. Also, there were small changes in wording to accommodate the particular conditions of each class. As a result the total points possible on each test varied between 100 and 110. Though 27 students comprise the study group, the total number of students who took the pre-test and post-test in PD3, 4 and 5 is 29. Two students from PD4 were also in PD5 because of AHS's small selection of course offerings. They had not failed PD4. In so far as evaluating the null hypothesis is concerned I have chosen to ignore that there were two repeat students. I believe this approach is valid because students come to my classroom with a wide variety of prior experiences yet I am evaluating them all according to the same standards. I consider these students to simply be persons with a high

degree of prior topic familiarity. Also, the second pre-test gives some information about long-term retention of PD information.

My hypothesis is that the instruction provided to students in PD3, 4 and 5 resulted in a gain of knowledge about the subject of microbiology as measured by written pre-tests and post-tests. Conversely then my null hypothesis is:

H_0 = there is no difference between the mean pre-test and post-test scores for students in the class, Tales From The Petri Dish.

n = 29

For the Pre -Test:	For the Post -Test:
Mean (x) = 27.0345	Mean = 66.3621
Standard Deviation (s_x) = 8.8297	Standard Deviation = 17.1604
First Quartile (Q_1) = 21	First Quartile = 54.5
Third Quartile (Q_3) = 31.5	Third Quartile = 77.25

According to Sincich (35) if the value obtained from the equation:

$$\frac{\text{Inter-Quartile Range}}{s_x}$$

is approximately equal to 1.34 then the data is normally distributed and a valid student t-test may be run. The values obtained were:

Pre-test = 1.19

Post-test = 1.33

Sincich considers these values sufficient to run a student t-test. This means that the null hypothesis can be rejected with a t value greater than 2.763 at the 0.01 confidence level and 28°of freedom. I ran a student t-test using the equation:

$$t = \frac{x2 - x1}{sx2 \bullet \sqrt{n}}$$

and found that t = 12.3415

Clearly students in the PD classes learned some of the material. A perusal of appendix Q will immediately explain the extremely high t value. Without exception student performance improved. While some of the poorer students nearly doubled their pre-test scores, increases by a factor between 2 and 3 were typical. The two students, #6 and #11, who repeated the class retained some of what they learned. Their second pre-test scores were higher than their first pre-test scores by an amount approximately equal to half of the increase between their first pre-test and post-test. Simply put, they remembered about half of what they'd learned a year later.

II. Demographic Information About Students In Tales From The Petri Dish

Only those students who took both the pre-test and post-test are included in my study group. As indicated in the introduction, students in PD are some of the better students at AHS but in a traditional high school they would probably rank much lower. The demographic data for individual PD

students (appendix R, S and T) supports many statements made in the introduction. The appendices of demographic data are several pages in length. I have supplied this much information to fill-out the readers mental picture of my study group. All data comes from the students' CA-60 paper files or the school's computerized attendance data base.

Appendix R lists information on PD students' age and Grade Point Averages (GPA) at various points in time. The GPA information has some trends worth noting. One, prior to attending AHS no students had an A or B average, seven of twenty-seven had C averages and the rest were failing or had D averages. So it is clear that even the better AHS students, those in the PD classes, have a prior history of poor academic achievement. Two, the GPA of all students increased at AHS prior to taking PD. Three, in comparing the GPA at AHS of the 24 PD students in the semesters before and during the PD class, thirteen declined while the others improved.

Appendix S shows attendance data. As stated previously only students who took both the pre-test and post-test are included in the study. The second and third column of appendix S show whether the student was present or absent from my class and does not take into account absences that were excused by the office. Some students that took both tests did not receive credit for one or more marking periods during the class due to poor attendance. Excused absences explain why several students with less than 80% attendance received credit. Columns 4 and 5 are included in case the reader should be interested in how long a particular student has attended AHS.

Appendix T lists grade level information and of the 18 students for whom numerical data was available, 11 were at least one grade level below their age group in at least one category at the time of the test. Of the remainder, five students were at grade level and two were above. Michigan Educational Assessment Program (MEAP) test results for secondary students are reported by category: a student is said to be at a Low, Moderate or Satisfactory level of achievement for their grade in school. Documentation that I reviewed indicated that a student who receives a Moderate rating in math is slightly below the standard set for students at this grade level. In reference to the information in appendix T, I assume this means students with Moderate or Low ratings were below grade level at the time of the test. PD students graduating in 1994, 1995 and 1996 are required by state law to achieve a certain score on the 10th grade math and reading tests in order to receive a state endorsed diploma. Satisfactory or Moderate ratings in reading or math allowed the student to receive the endorsement. A reading of the MEAP results column in appendix T shows few students with any Satisfactory ratings. The only two students who had two Satisfactories achieved this in 7th grade.

While 37% of the whole student body was in TO at least once in the last year, the rate was higher in PD5 students since seven out of thirteen or 54% of these students were in TO. In the introduction a breakdown of the frequency of TO referrals for all students was shown. No PD students were in the group that received 15 or more TO referrals but were almost evenly split between

the two groups with fewer referrals. The categories of offense resulting in referral in decreasing order of occurrence include disturbing class, smoking, not working in class, skipping, and drug possession. TO data is unavailable for PD3 and 4 students.

In terms of their behavior, PD students are typical of the majority of AHS students. They are not chronically disruptive. The offenses listed as causes for referral are not violent or criminal actions or even unusual behavior for adolescents. I see these students as making poor behavior choices that are ultimately self-defeating.

PD students are typical of the student body as a whole in the dramatic increase in GPA at AHS as compared with performance at their sending school. I had hoped to see an increase in the GPA of PD students while they were in the class so that I might say their motivation had increased but no clear trend emerges from that data. Based on my experience, PD students are slightly better than the majority of AHS students in attendance though I am sure most of the percentages listed in the third column of appendix S would be unacceptable in a traditional high school. With regard to academic abilities as measured by standardized testing instruments, PD students probably score higher than the average AHS student. Yet still about half the PD students have skill deficits and most of the others have only average abilities.

As stated at the beginning of this section the above information is intended to fill-out the readers mental picture of my study group. I think it is

important for the reader to remember that while PD students might be better than the average alternative student in some areas, they are still at-risk youth. As a sidelight and possibly in reference to why these students are at-risk, the two students with superior standardized test scores were referred to TO in 95-96 five or more times. Only 25 students in the whole school were referred to TO more often.

III. Analysis of Pre-Class and Post-Class Student Interviews

What follows is an analysis of student interviews conducted in PD5. Rather than include the exceedingly lengthy verbatim transcript of student responses, I have attempted to convey the spirit of their statements. Many quotes are included as well as paraphrasings. Where it seemed appropriate for clarity, student responses are in a list format. At other times lists of student responses may be in paragraph form.

The pre-class and post-class interview instruments are contained in appendix K. In the following analysis each question of the instrument is referred to as an Item and is in a form relatively unaltered from that found on the post-class instrument. I did not include the pre-class questions in the analysis, only the pre-class student responses. The reader may generally assume that information given is from the post-class interviews unless it is indicated otherwise in the text.

In selecting the students to be interviewed I predicted their final standing in the class based on my experience with them in previous classes. I

anticipated one would finish near the top, another at the bottom and the last two in the middle of ranking by grade. Unfortunately the students I selected were skewed toward the high end of the class. The class size was fifteen and students I have designated here as A, B, D and C finished 1, 3, 5 and 10 respectively in grade ranking. The letters are arbitrarily assigned for the purpose of clarity while also protecting student confidentiality. I selected three males and one female because this reflects the male/female ratio of the class and AHS as a whole.

Students were encouraged to make extensive comments. To this end, I did not pursue some points with students for fear of intimidating them or altering their answers.

Items 1 and 2 were asked to put the subjects at ease. The information requested was name, age, sex, place of birth, place of residence and name of the class.

Item 3 - Was the class what you expected it to be?

Three students said yes and one said no but all of them reported suprise at the inclusion of some topics in the course. Two of four students reported suprise at the connection between food and microbiology. It made sense to them now but they hadn't considered it before. A third student said he expected only to cover bacteria and foods. The post-class interview responses of all four students imply that they had expected to study bacteria

and other topics from the beginning. In their pre-class interview, however, bacteria and these other topics were not mentioned. The same three students said in the pre-class interview that they expected to use microscopes, do some experiments and go outside to collect samples. I can think of two reasons for this discrepancy between pre-class and post-class interviews. One, the students had a vague understanding of what sorts of things might be included in a microbiology course but lacked the vocabulary to verbalize what they did know. Two, the students were not being entirely honest in their post-class responses to please me. Because I made it clear to students that these interviews had no affect on their grades, I tend to believe the discrepancy between responses is caused by a combination of both things.

Item 4 - Are you glad that you chose this class or do you wish you had taken another?

All four students were positive about taking the class. Students A and C said "it was cool." Another said he liked learning things that were entirely new for him. The fourth reported that he was considering pursuing field oriented plant pathology in college. Three of the four students had chosen the class because they had had me for class previously and/or "it sounded like fun." The fourth student (who was one of the two students who described PD5 as "cool") had not chosen the class and was in fact quite negative at the beginning because he had strongly preferred a different science elective.

Item 5 - List 3-5 things you did in this class.

Below is a list of student responses. The numbers in parentheses indicate how many interviewees mentioned that response.

Enzyme experiments

Made a fungus garden (2)

Learned a lot about fungi

Went on many field trips (2) "to see how microbiology is used in the real world"

Went on field trip to winery

Went on field trip to "sample.. Chestnut Blight from the American Chestnut"

Made beer (2)

Made yogurt (2)

Made sauerkraut

Learned sterile technique

Learned how to autoclave

Grew bacteria

Counted bacteria

Identified bacteria

Tested antibiotic resistance [of bacteria]

Answers to this question give an indication of what was most memorable for students and there is a wide variety of responses. Perhaps this indicates that the range of experiences structured into the class offers adequate

opportunities for student engagement. In comparison, the pre-class interview responses were:

No idea

Experiments

Worksheets

Look through microscopes (2)

Grow and make our own penicillin

Collect samples outside

It seems the students have gained a broader, and at the same time, a more detailed conception about the field of microbiology.

Item 6 - Were you excited and/or interested in the following parts of the class? Experiments / Field Trips / Information

In general, responses from two students (B & C) indicated they felt more interest than excitement (these students preferred the experiments over field trips and information), while the other two students felt more excitement. Students D and B spoke at length. Student B reported this was because he liked the "hands-on" experiments, getting to "set-up experiments", and because he felt that he learned a lot. Part of what he liked about learning was seeing "what the facts were" and the example he gave was making beer. "I didn't know beer was made anything like that, with yeast and all that stuff. I thought you took some barley and ground it up [then added water and alcohol] from somewhere else. I didn't know it was made by the

yeast and all that." Student C also reported that she had learned new facts about some things. The example she gave was the scale and technology of commercial winemaking. She had been half-expecting to see people crushing grapes barefooted.

As for the students who said they experienced a higher level of excitement than interest, student D said this stemmed from the field trips because "I get out and actually do it and visit the place where it's done." Also, both students A and D were very clear that the notes and other information were interesting because it aided their understanding. Student A said "The notes helped me understand.... It made everything else interesting so in itself it was interesting."

In comparison with their pre-class interview responses, students B and C showed a high degree of consistency between expectations and outcome in all three parts of the class. Students A and D, however, reported changes in attitude. At the pre-class interview student A had to be prompted twice to describe his expectations before giving a sincere answer and that was a very lukewarm endorsement of my ability to make learning "kinda cool." Student D who was positive about the value of notes and information at the post-class interview said at the pre-class interview that he hoped "they're not too lame."

Item 7 - What is microbiology and what is usually considered to be part of it?

I treated this as a two-part question with students and have done the

same here. I have paired the pre-class (first line) and post-class interview (second line) responses of each student to emphasize any changes in their definition of the word microbiology.

A-Study of small things, microscopic size, bacteria and single cell organisms

A- The study of micro-organisms.

B-"Something to do with microscopes and microscopic animals."

B-The study of bacteria, fungi and viruses.

C-"It's where you're looking under a microscope."

C-The study of plant diseases, no, it's the study of life under a microscope."

D- Study of things you can't see with the naked eye, "algae and stuff like that."

D-"I believe that's ....the food thing", studying the micro-organisms involved. I asked student D if there were anything else and he responded by vaguely describing plant pathology and medical microbiology before finally recalling and repeating the same definition he had given at the pre-class interview.

It seems that the later definitions given by students A and B are no worse or slightly better than the first. However, the other two students leave the impression that they are confused and I find this somewhat disturbing. Their responses may reflect the fact that I did not overtly emphasize a particular definition of the word and need to do that in the future.

Student responses to the second portion of the question were varied in both length and content. Student C said "[mostly] the stuff we did in class" while the others rattled off accurate examples of how microbiology is used including mushroom culture, making medical diagnoses, food safety and

wastewater treatment. Because of a lack of academic success I think that "alternative" students may feel more comfortable talking around a word rather than defining it. All things considered I am satisfied that these students have a fuller understanding of what microbiology is because of how they discussed their examples.

Item 8 - What is a microbe?

I asked this question with the word microbe instead of micro-organism, to three of the students. One said that the word meant the same as micro-organism, another said they must have been absent that day and the third said nothing. I asked the latter two if it would help if I used the word micro-organism instead and both said it would. I did not think to pursue this further, in retrospect I wish I had because it is an odd discrepancy that I would like to understand. I assumed the words were as interchangeable for students as they are for me. This is a point that I need to clarify in the next PD.

Item 8 a -List types [of microbes]

Bacteria (4)

Fungi (4)

Viruses (3)

Yeast

Algae

Plankton

Very small insects

Cell Wall

Enzymes

These results are like a good news/bad news story. The good news is that students gave the names of the two or three groups of microbes we spent the most time on. However, the last three names given show an area that I will need to clarify in the next PD. Student D suggested very small insects and cell wall as microbes and in a way these items are consistent with his definition of microbiology since one cannot see either with the naked eye. Student A suggested enzymes yet did very well on the post-test. The enzyme notes do not explicitly say that enzymes are not a lifeform, only that they were originally discovered in living things. Perhaps he interpreted this thinking that bacteria are also found in living things. Also, the series of enzyme experiments in the PD introduction received less time but otherwise comparable emphasis as the general information about fungus or bacteria. In the next PD it will be useful to be more explicit about the definitions of microbiology, microbes, micro-organisms and enzymes.

Item 8 b -List characteristics [of microbes]

Student responses were quite varied and included the small size of individual microbes, that some are single-celled organisms (bacteria) and some are not (fungi), that they absorb their food source through the cellular exterior, that some are aerobes and some are anaerobes, that fungi produce spores and are made of hyphae that get food and attach [to a substrate], that

they have identifiable shapes [and all the things we looked at in describing colony morphology]. Each student said two or three of these things, but two statements stand out for me. Student B said "That's one thing I learned in this class. I thought they were all basically the same thing but they really have a lot of different characteristics." I was pleased by his response seeing it as a synthesis of information. Student C said "most infect something." I am fairly sure that I said no such thing if for no other reason than that it is probably not true. However, her response shows me another possible misconception that needs to be explicitly addressed next time.

Responses in the pre-class interview emphasized the small size of microbes and to a lesser extent that microbes are unicellular. My conclusion then is that there was growth in the richness and variety of student understanding.

Item 9 - _____, who uses microbiology and how?

I asked this as three separate questions where the underlined words shown below would be inserted in the blank. Under each are a sample of the responses.

Item 9a - <u>In General</u>

All post-class interview responses were in the area of medicine and health. They included disease identification, research, anyone who works in a hospital or around [the young of any species], blood analysis for cell counts and drugs, producing antibiotics, culturing pathogens to test the value of

antibiotics.

The pre-class responses were: marine biologists, the Upjohn pharmaceutical company, hospital medical technology labs, biologists, disease control officers, doctors and "in wastewater treatment (2)". If anything there was somewhat more variety in the pre-class responses. This is to be expected because I did not include any sort of general survey of the uses of microbiology outside of the topics the class emphasizes. The question was included simply to see what students would say.

Item 9b - In making or studying foods

One student listed brand names but the others simply said producers of some or all of the four foods that we made plus cheese and bread as well as mushroom growers. In answering how microbiology is used in producing these foods, all four students gave detailed and accurate examples from either the Campbell's Fresh or winery field trips. In the pre-class interview two students could not think of any uses while another said micro-organisms cause food to decay and the last student talked vaguely about how he thought companies produced sour cream using microbes and that all food producing companies would check to see if foods are contaminated. Again, there is a richness to the post-class interview answers that was absent before.

Item 9c - In studying plant diseases

In the post-class interview, Student C could not think of who or how anyone might use microbiology in studying plant diseases other than to repeat that professors from M.S.U. had come to see the very large American

Chestnut at the edge of town. Students A, B an D all suggested various persons in the agricultural field - farmers (2), [agronomist], tree farmers, F.D.A. vegetable inspectors and Michigan D.N.R. preserve managers charged with the responsibility of monitoring general forest health. One particularly perceptive answer was that importers should be aware of microbiology so that they wouldn't bring in a different microbe that would "wipe out a whole species of ....whatever." In the pre-class responses two students had nothing to say, a third thought a botanist might use microbiology and student D knew that "mushrooms that grow off the sides of trees is a disease." He also wondered if maybe mosses weren't also a plant disease.

Given that foods and plant pathology were the two special areas emphasized in the class I find the growth in their answers for these questions satisfactory. In the next PD it would be beneficial to students to include in the class introduction a survey of the whole field of microbiology.

Item 10 - What ideas did you learn from this class and/or How has taking this class changed your view of the world?

I found these student response to be the most gratifying. Assuming the students were sincere, and I have no proof to the contrary, they gained some profound insights from what they studied. Student A described at length how he made the connection between hearing about something (i.e., a place, invisible microbes) and having it truly exist for him. "if I go over there and grab that doorknob, what's gonna be on it [so] you realize there's more to the

world than just what you can see. Maybe it might not just be the small things you can't see [it might be] other cities, other countries."

Student B reiterated the caution that he felt an importer should exercise and added that if he were an importer he wouldn't want to be responsible for bringing about the end of a plant, such as corn, that is so important to the survival of millions. On a more personal level he added "I have a baby on the way [so I] go through a little more cleaning of stuff because bacteria can be spread so easily....you touch something one place and touch another place and it's like there!"

Student C reported that the class experience had affected her food choices. For example she won't eat sauerkraut now because it is unrefrigerated so long as it ferments and to her that seems unsafe even though she knows better. On the positive side she says she is more open to trying some homemade things, like ginger ale. Also, student C mentioned gaining a new appreciation of the value of the American Chestnut.

Student D discussed his heightened awareness of the value and vulnerability of plants - "Nobody wants a barren ugly world....[I just realized] how susceptible trees are." in addition he was impressed by "how important plants and micro-organisms are to the economic system. As in the food thing, if we didn't have certain types of bacteria or fungus we wouldn't have certain types of food."

These comments are more thoughtful than what I am accustomed to hearing from AHS students. In their pre-class responses students either had

no idea what to answer or suggested things that would more appropriately be called skills. It seems valid to attribute some positive impact by the class on the students level of awareness.

Item 11 - Name 3-5 skills you have learned from this class.

Basically post-class responses to this item were a reiteration of what was said for Item 5. Since their responses were stated as skills not as things or activities, the interviewees were making a distinction that I feel shows they understand the word as it relates to microbiological skills. I would have felt they had missed something if they had not made that discrimination. One student maintained he had never used a microscope before. Responses continued to affirm the trend of richer and more varied answers as compared with pre-class responses.

Item 12 - Did you learn any skills or information from this class that you will be able to use in:

Item 12 a - the real world

Responses centered around possible uses in the home or garden such as improving hygiene for self and/or infants, food production and dealing with garden diseases. One response I liked was "how to treat the world" in reference to not transporting new pathogens or exotic species. In the pre-class interview student D was the only one to respond and he said others could find cures for the most important diseases.

Item 12 b - a future job

Three students could see potential applications of PD skills and information in the careers in which they are interested The fourth, student D, says he is interested in pursuing plant pathology as a career. In the pre-class interview student A thought it was a remote possibility and the other three could see no applications in the careers they wanted to pursue.

Item 12 c - a later science class

In the pre-class interview three of the students said that if they went to college and took biology classes, then the PD skills and information would be valuable. In the post-class interview these same three students indicated a greater willingness to include, or in one case emphasize, biology classes if they attended college. Student D, the student who is now interested in pursuing plant pathology, had no response at the pre-class interview. He believes having the PD class on his high school transcript will be useful and that the skills and information will be useful in his course work.

In all three areas of Item 12 post-class responses were much greater in number. I would assume from this that for the most part students see usefulness and value to what they learned in the class.

Item 13 - Do you think it is valuable to simply have general knowledge (such as how cheese is made) that you may not use in the real world, a future job or a later science class?

Students B, C and D said yes to this question in the pre-class interview

and reported no change in their attitudes after the class. However, as with responses to several other questions, post-class interview responses were more lengthy and descriptive. I am not sure why that is, perhaps they became more comfortable with me or perceive the PD classroom as a place where they can "be smart" with less criticism from others. Student A's pre-class interview response was "not really" His post-class interview response was a lengthy explanation of knowledge being power and how that might apply in his life. I don't believe he learned all of that from studying microbiology, so I assume he is just more comfortable.

Item 14 - Now that you have taken this class would you consider a career in science or a related field?

As in Item 7, the pre- and post-class responses of each student are paired. After each set of responses are comments regarding my interpretation of the effect of the PD class on their attitudes toward future employment.

A-" Yes, I want to be an astrophysicist."

A - Unchanged

Student A is a freshman and quite intelligent but he has very low self-esteem. His mother took an evening astronomy class at a local two-year college and usually brought him along. The instructor treated student A well and used him in classroom demonstrations. I think his interest in astrophysics stems more from the respectful treatment he received than interest in the subject. Student A does very well in science classes and may very well work in science

or a related field if he sorts out his difficulties but PD does not appear to have caused a significant alteration of his attitudes toward future employment.

B - "Probably not"

B - "Not immediately, but down the line [if things came together] I'd definitely at least think about it."

Student B was a sophomore this past year, expects the birth of his first child this summer, is a gang member and is currently on an electronic tether for firearms charges dating back two years. Student B is also intelligent, perceptive, polite and a pleasure to be around. Given the difficult situation in which he currently finds himself, B may be considered as doing well in the future by responsibly raising the child he will soon have and supporting it with legitimate employment. I do believe that B was sincere in his post-class comments and I think PD may have had some role in that change of attitude.

C - "No, I want to go into computers" [as a secretary]

C - Still most interested in secretarial but would now at least consider it especially if the two could be combined.

Student C will probably graduate next year and become a secretary. I do not see that PD has had a significant impact on her career choices.

D - Yes, I might work in a wastewater treatment plant or in the factory at UpJohns.

D - Is interested in pursuing plant pathology as a career

While Student D expresses these desires with reasonable confidence of their attainability I am not so confident. His verbal skills are poor and his level of

motivation is at times quite low. Also, his attraction to plant pathology may have more to do with a perception of it as a field where one spends considerable time outdoors than a deep interest in the subject. On the positive side however, he has usually done well in science classes, as he did in PD, and he did graduate this year. I will say, with reservations, that participation in PD has affected his choice of potential careers.

Item 15 - In the pre-interview you said (*Answers varied*) about science classes in general, has this class changed your feelings about that or how do you feel about this class compared to others you've taken?

All students reported no attitudinal change. Students A, B and D had positive feelings about science classes before and after PD. Student C is still weakly positive about science classes.

CHAPTER 5

CONCLUSIONS

What Was Effective In TALES FROM THE PETRI DISH?

As mentioned earlier, I believe the results of the student evaluations are accurate reflections of student opinion. In the following discussion of what was effective and what needs improvement I will frequently refer to the ratings or values that students gave to the experiments and field trips. The student interviews are also an important source for this discussion.

I asked all PD5 students to rank, in order of preference, the five main units of the PD class after they were finished evaluating the labs and field trips. In retrospect, I think it would have been valuable to treat enzymes as a unit so that it would be included in this ranking. The ranking from most to least liked was:

Food Microbiology

Fungus

Bacteri

Plant Pathology

Sterile Technique

One of the most successful aspects of the PD classes is how much the students learned. Even students who failed the class or had poor attendance improved their scores and most improved by a factor of two or three. Contrasted with pre-test/post-test score comparisons from other classes I have taught at AHS, these are phenomenal gains. In nearly all interview questions that were intended to measure the students concept of the size and contents of the field of microbiology, responses in the post-class interviews were longer and more detailed than those of the pre-class interview. I have no hard data to back it up, but my feeling is that most PD students felt pride in themselves for learning so much about a new and challenging subject. It is possible to teach a quality microbiology class to at-risk teens and it is possible for them to succeed in the class. I have no doubt that some of the special atmosphere of these PD classes happened because the students knew this was important to me so it became important to them. The challenge will be to replicate that sense of importance in future PD classes without the motivation of thesis research.

Positive attitudinal changes were brought about by the class. In responses to Item 12 b students indicated they could now see some possible applications of microbiology in future jobs where they saw none before. In item 14 two of four students definitely exhibited a positive change toward consideration of science or a related field as a career. Described in Item 10 were the insights leading to a new world view that students attributed to the PD class. Pleasing on a personal and professional level is the concern shown

by students for the American Chestnut and it's struggle with chestnut blight. I have rarely stimulated as much concern about any environmental issue in other classes. All of the interview students mentioned the Chestnut or blight in one context or another, including student B's thoughts about the precautions that an importer should take.

I am generally satisfied with the selection and arrangement of units in the class. I feel that it worked well to lay groundwork by covering enzymes and sterile technique at the beginning of class. Even though some students may think that enzymes are organisms, most students do seem to understand the importance of the role that enzymes play in life-sustaining microbial processes. Though learning sterile technique was the least liked unit I can hardly drop it. Perhaps ways to make it more challenging should be found. I feel some disappointment that plant pathology was less well liked than bacteria and fungi. However, considering the lack of closure on some of the experiments, I am not surprised. Certainly this is one area of the class that requires fine tuning.

Brewing beer was probably one of the smarter choices that I made in designing the course. It ranked first in student interest and did well in the other two categories. Beermaking was one of four things (out of a total of 15) to be mentioned by two students for Item 5. Brewing probably has much to do with food microbiology being the most-liked unit.

Students reported that they both liked and learned from the field trips. Results of the student evaluations show that out of the twelve values for the

field trips (three questions multiplied by four field trips) five were above 0.90 and the rest were 0.83 or above. This means that not only was the interest high but that in all cases students felt they understood what was going on and that it helped them understand classroom material. What I might typically hear from students about field trips in any other class is that they are just happy to "get out of class." In PD, students did indeed "get out of class" but they were writing reports about the trips so their interest was not simply from the fun. The field trips were mentioned in responses to most questions during the student interviews. Effects mentioned there range from challenging student preconceptions to stimulating student D's interest in plant pathology as a career to seeing unique things for the first time.

Prior to PD5 I had never done interviews with students. The time required for all phases of the process - preparation, interviews, extracting data and interpreting data - is considerable. However, I learned things from the interviews that I would not have known otherwise. These include factual misconceptions such as the students who listed enzymes, cell wall and very small insects as types of microbes. Clearly these are points to address in the next PD. Also, through the interviews I get a much better sense of how my students think and what parts of the class are significant to them. Another aspect of the interviews is positive reinforcement of the instructor. On the whole, what went on in the PD class was good and the interviews reflect that.

Compared with my other upper-level class, Stream Team, PD requires a much greater amount of planning and making arrangements. But with

some exceptions most of the things I tried to do with the class worked well enough to be called successful. For example, arrangements for the four field trips, which only consumed four of the 80+ days of class, include finding suitable destinations that tied into class content, scheduling times and dates with the people at the destination, arranging transportation, permission slips, writing report forms, preparing for discussions about the trip before and after it occurs. But as I discussed in a previous paragraph the field trips were a very successful aspect of the class.

I found it exciting to explore what may be new territory in regular high school classrooms and certainly is in alternative classrooms - teaching plant pathology using experiments about chestnut blight and the resistance of cucumbers to disease.

What Needed Improvement In TALES FROM THE PETRI DISH?

As stated above, the food microbiology unit was ranked by students as the one they most liked and that brewing beer was the most popular of the four foods produced. It is less clear which of the other foods is the least popular. There are statements in the student interviews that could be interpreted as for or against producing yogurt, ginger ale and sauerkraut. In the student evaluations making ginger ale is 0.17 to 0.19 points ahead of making yogurt and sauerkraut. At the Microbiology Meal of PD4 the sauerkraut was uneaten, ginger ale was tolerated and the nearly all of the

yogurt was consumed. I suspect that the context had more to do with these inconsistencies than the foods themselves.

There are three possible ways to alter this situation. One, don't produce any foods except beer. I don't like this solution because I think the variety of products is valuable for student understanding and allows greater opportunities for students to find something that appeals to them. Two, substitute other foods for yogurt, ginger ale and sauerkraut. Beyond cheese and pickles, I am not sure that there are other foods produced by microbes which would be familiar to my students. Also, both cheese and pickles require long processing times and given the difficulties already experienced with experiments that span days, I don't need to add another long-term project. Three, change the context in which these three foods are presented. This is the solution that most appeals to me is since it builds from what I already have in place. Future classes could have fun by making semi-recreational time in class to taste foods as soon as they are ready. For example, we could grill hot dogs and bratwursts to put sauerkraut on or use a variety of toppings to mix in with yogurt. I did not overtly schedule this time in previously because I assumed that the students would automatically take care of eating the foods.

By far the greatest difficulties in the class were issues of time. There are three aspects of this that I would like to address. One, some of the plant pathology experiments were not completed because not enough time was left at the end of the course. Two, some of the projects that involved leaving

materials for a period of days after the initial set-up were not returned to in a timely manner. Three, considering all factors, what length of class period and what total class length should I request the next time PD is in the AHS schedule.

Since PD5 did not include the unit on tissue culture and was taught over a period of sixteen weeks as compared to the nine weeks allowed for PD3 and 4, one would assume that the plant pathology experiments should have been completed. The primary reason why they were not is that I asked for much more in the way of written work from the PD5 class, especially in the units of the class prior to food microbiology. Probably five or six times as much written work was required in PD5 as compared to PD3 or 4. That was especially time consuming given the skill levels of my students. Also, many of the papers were revised by students and resubmitted. The question must be asked: Was all the writing worth-while and was it worth not completing some of the plant pathology experiments? I do believe writing by students is worth-while in all subjects. However, I don't think the quantity of writing requested should prevent the timely completion of experiments. I believe that the writing probably led to greater student understanding, but I have no data to support that belief. Also, I imagine that the amount of writing required and that I asked for revisions is part of why students felt that the course was important to me (as discussed above). I do not intend to drop plant pathology from future versions of PD so writing in the earlier portions of the class must be curtailed somewhat. Additionally, I could drop one or

two of the less popular and/or less effective labs intended to introduce enzymes and basic information about bacteria.

The problem of revisiting experiments days after the initial set-up does not have so obvious a solution. Experimental projects that have a higher degree of interest for the students, such as brewing, were less often overlooked. For my part, I tended to focus solely on the experiment at hand. Perhaps greater experience with these experiments will better enable me to step back to see the big picture and arrange for students to revisit the experiments. Another solution for the near term would be to designate a student or students whose responsibility it would be to keep track of all the experiments currently being conducted.

Next year AHS will be in a different building and the program has been downsized from nine to six teachers and by 20 or more students. It is not clear yet whether we will be able to offer anything beyond the basic two years of biology and physical science required for graduation. Also, the trend at AHS over the last few years has been away from block classes. This past year I was the only teacher to have block classes all year and next fall I won't have any because it makes scheduling students difficult. The flexibility allowed by having 110 minutes with students each day has been an important part of PD. The nine week length of PD3 and 4 was too short for all that I tried to include in the class. With good organization and a reduction in the number of labs in the first portion of the class, I think it may be possible to offer PD as a one-semester class that meets one hour per day five days a week.

The most basic question for any educator must be "Is this good for the student?" because nothing else matters as much. Tales From The Petri Dish was definitely good for the students in my classes. They saw things that were entirely new to them, they were challenged by the material and they had enough opportunities with all the different experiments to find something they liked. Certainly there are portions that could be improved but I think it is possible to meaningfully teach microbiology to at-risk high school students. One young mother who was not an excellent student and missed 24 days during the class said she was taking the post-test "You ought to be proud of yourself because I feel like I really learned something in this class. I usually don't feel that way when I'm taking a science test."

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APPENDICES

APPENDIX A

MICROBIOLOGY IS... & STERILE TECHNIQUE

MICROBIOLOGY IS.....
1-The study of organisms visible under a microscope and anything else about them including their culture, economic importance, pathogenicity, etc.
2-Microorganism and microbe mean the same thing - organisms not visible to the naked eye
3-The Main Groups of Microbes include the following

- Bacteria
- Fungi
- Algae
- Viruses
- Protozoa

STERILE TECHNIQUE IS .....
Number 1 requirement to keep you healthy and for consistent success in microbiology (by preventing contamination of the desired microbes with undesirable organisms)

BASIC CONCEPTS
Usually we are trying to have only the stuff we are studying grow in a container

There are gazillions of bacteria and fungal spores in the air and on everything (especially your hands) just waiting to "hatch out", that's a good and bad thing
GOOD BAD

Heat
Flaming - passing an object through flame until it is red-hot allow to cool
Boiling - in rolling type boil if all surfaces open for 10 min.
Baking
Autoclaving - pressure cooker, dangerous if handled improperly

Sterilizing Liquids
Alcohol (70%)
Bleach in low concentrations (5-15%)

Inverting Dishes -
Invert means to turn upside down
The purpose is to keep liquid that condenses away from media surface

Removing A Petri Dish Lid
Open it only as far as is needed but far enough that you can safely work on the dish
When it must be entirely lifted off the cover should be held above the plate

Petri Dish Transfer
Flame the NiChrome Loop, Allow to cool
Dip loop slightly into what's being transferred
Touch loop to second plate
Flame loop, repeat, repeat, repeat

Reducing Exposure
Keep things covered -particles in air (including bacteria) most often fall down
Cut down the flow of air for example - outside air to a more sterile area
Most of all think about what you are doing and take appropriate precautions - SAFETY FIRST

WORKBENCH HYGIENE
Cleaning area - use paper towel or sponge to spread a 10% chlorox solution, before & after using a tabletop
Discoloration of clothes due to contact with liquid bleach is possible if you're not careful

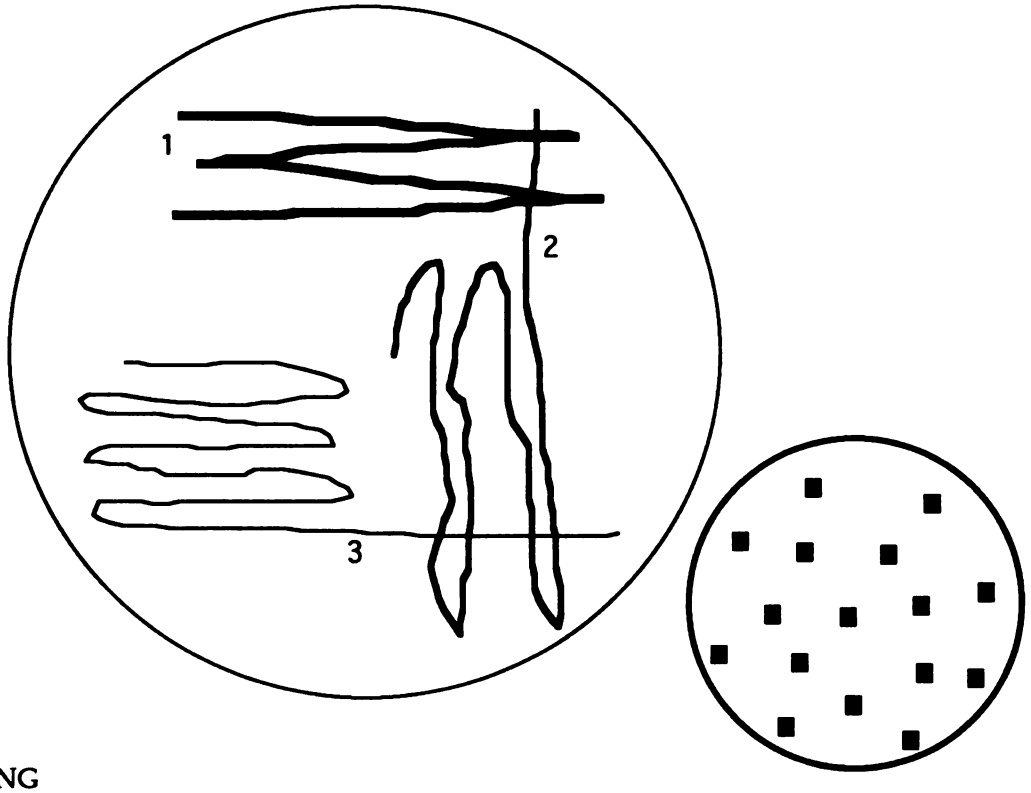
Proper disposal- anything we make with bacteria or fungus should probably be placed in sealed "BIO-HAZARD" bags

Dangers of breathing -BE CONSCIOUS of what we're working w/ chemicals, fumes, bacteria may be inhaled, get past immune defenses and cause a problem

Eating/drinking - not at tables or maybe even in room when we are doing an experiment

WORKING ON MEDIA
Media is the name for the material on/in which a microbe is grown
-The media used in petri dishes is generally a gelatin-like substance (agar) with various additives such as nutrients, pH indicators and much more!!!!
-A liquid media (called broth) may also be used.

STREAKING
-to just transfer (1)
-to isolate one organism (1-2-3)



PLACING
-to fit several transfers onto one dish

APPENDIX B

ENZYMES NOTES

Enzymes:

-they DO things by helping chemical reactions happen lots faster and easier usually without enzymes the reaction would require high temperatures &/or extreme pHs

-enzymes are not used up in the chemical reactions so a little enzyme helps lots and lots (but they do wear out in weeks or months)

-enzymes are made by and were originally discovered working in organisms but can work outside organisms
[Louis Pasteur yeast fermentation (glucose to ethanol & CO_2)]

-enzymes are found in all organisms, life is not possible without them BUT ENZYMES THEMSELVES ARE NOT ALIVE

-most enzymes only work to make just 1 chemical reaction happen (there are 19 chemical steps in changing glucose to CO_2 and water, each step requires it's own enzyme)

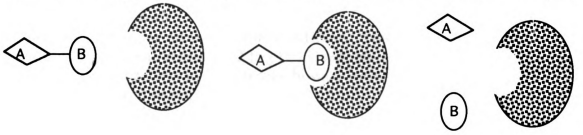
-they are a type of protein but they are not a nutrient and are not digested

-heat will "denature" or wreck an enzyme

-names of enzymes usually end in "-ase"

-concentrations of enzyme and substrate affect the speed of enzyme reactions as do temperature, pH and the presence of inhibitors

Substrate + Enzyme --> Enzyme-Substrate Complex --> Products + Enzyme



APPENDIX C

BACTERIA NOTES

1) WHAT THEY ARE

a-probably the first form of life on the planet

b-they have no internal membranes, insides of cell very simply organized

c-some are photosynthetic, most "eat" living or dead unicellular or multicellular organisms

d-they eat by chemical reactions, they will use any reaction that produces energy and only reactions that produce energy

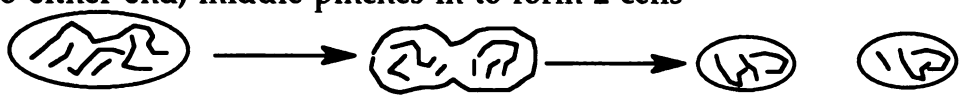
	(sugar)					
Example	Glucose	+ Oxygen	-->	Carbon Dioxide	+ Water	+ Energy
	$C_6H_{12}O_6$			CO_2	H_2O	

in digesting sugars, bacteria gets C, H & O to "build" new cell parts and the energy to do it with

2)Reproduction

a-reproduce very fast, average doubling time is 20 minutes.

b-reproduce by simple cell division. Contents of cell are split evenly, pushed to either end, middle pinches in to form 2 cells



c-each species of Bact has a maximum cell size, when it gets that large it divides

d-bacteria can also form spores to survive heat, drought, poisons, etc.

3) WHERE THEY ARE

a-Bacteria live under all imaginable conditions, bacteria are everywhere Antarctica to thermal vents, pH 2 to pH 13, soil, air, water, etc

b-the weight of bacteria in the average human body is about equal to the weight of 12 oz. can of pop

4) IDENTIFYING BACTERIA

a-AEROBES take in oxygen to "breathe"

ANAEROBES "breathe" other gases - methane, ammonia, etc.

b-CATALASE or REACTION

-amount of bubbles produced shows how much enzyme is present

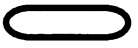
c-Types

a-Shapes

cocci (spheres)

spirilli (corkscrews)

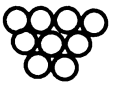
bacilli (rods)



Grouping

chains

sheets



clusters

c-GRAM STAIN

-describes the ability of a bacteria to hold gram stain in their cell membrane

-positive bacteria hold the stain, negative do not

-depends on 2 things -thickness and material of the membrane

-pore size

-generally different antibiotics would be used on each group

-UV light makes free radicals, which tend to break things apart (example: collagen fibers in skin, as they are broken skin sags & gets leathery - aging!)

-the enzyme makes free radicals safe to organisms

-Hydrogen Peroxide is not in body, it just acts like free radicals so enzyme attacks it and makes it into water and oxygen

e-COLONY APPEARANCE

-one single bacteria after reproducing for a time will become a group of several million that is visible

-visible characteristics can be observed and classified (see Cultural Characteristics of Bacteria chart)

Comparing How You Study MacroOrganisms & Micro-organisms

Macro-organisms	Micro-organisms
-body and behavior are important	-metabolism & physiology are imp
-genetic exchange is restricted	-genetic exchange is less restricted
-can see their environment	-exact conditions of their environment is hard to see
-can easily see differences	-must often "test" for differences

HOW HARD IT IS TO KNOW SOMETHING ABOUT AN ORGANISM

MICRO-ORGANISMS — MACRO-ORGANISMS

easier ↓

DISTRIBUTION
HABITAT
INTERACTION
GENE POOL
GENETICS
GENE EXPRESSION

↑ easier

Appendix D

FUNGUS NOTES

STRUCTURE

Hypha are the long strings of fungus cells that do the regular job of living for a fungus such as eating and growing.

The mycelium is a group of hypha where there are enough hypha to see with the naked eye.

FUNGI have a cell wall made of CHITIN, this protein is most often found in one other group of organisms -> INSECTS. Their hard shells or exoskeletons are also made of chitin.

EUKARYOTES - fungi have internal membranes, unlike bacteria. Some types though do not have separations between cells.

TYPES - Examples of fungi include

Yeast	Slime molds
Mushrooms	Puffballs
Molds	Most plant diseases
Bracket fungi	

REPRODUCTION AND GROWTH

- A new mycelium can start growing in a new places by the transfer of spores or pieces of hyphae.
- Spores have hard thick walls so they can survive bad conditions. Hyphae cannot.
- Spores can be produced by sexual or asexual means. Yeasts asexually reproduce by budding.
- Growth is by simple cell division.

NUTRITION

- Though they may look like plants, the fungus is eating it's food by making acids and enzymes to dissolve the food source and then absorb the food through the cell wall and cell membrane.
-Fungi eat living (parasite) or non-living (saprophyte) materials

-Because of their ability to make strange and powerful chemicals, fungi can live on and eat many strange things.

Appendix E

FOOD MICROBIOLOGY NOTES

The purpose of both fermentation and respiration for micro-organisms is to produce energy. They happen to produce by-products that we like - alcohol, acids, carbon dioxide, curds, etc.

Most foods produced using microbes involve fermentation.
Most beverages produced using microbes involve fermentation.
Fermentation does not always results in the production of alcohol.

When a micro-organism is used to produce a food or beverage, it "eats" raw material(s) using enzymes and then makes a desired product
For Example: Lactobacilli use lactase to digest lactose and are used by the food industry to make sauerkraut (proprionic acid) and yogurt (curds).

All production of beer and wine depends on the many species of the YEAST fungus.

Microbes eat by any chemical reactions that produces energy and only reactions that produce energy in digesting sugars, microbes gets C, H & O to "build" new cell parts and the energy to do it with

	(sugar)			
Example	Glucose + Oxygen -->	Carbon Dioxide	+ Water	+ Energy
	$C_6H_{12}O_6$	CO_2	H_2O	

SEEN THIS BEFORE????????
You should remember it from the bacteria notes. This is the chemical formula for respiration but fermentations work in almost the same way. Other things will take the place of the Oxygen and maybe for the Water and Carbon Dioxide. Sooooo.....

Fermentation doesn't require Oxygen (anaerobic)
Respiration does require Oxygen (aerobic)

Photosynthesis and respiration are the ~reverses of the same chemical process/equation. See if you can write the photosynthesis equation!!!!!

As we go through the experiments describe below **which micro-organisms** are **doing what with what** and how that **results** in the desired product.
a)Cabbage and Salt into Sauerkraut
b)Hops, Malted Barley & Water into Beer
c)Granulated Sugar, Lemon, Ginger, Tartar into Ginger Ale
d) Milk into Yogurt

Appendix F

PLANT PATHOLOGY

DO YOU HAVE ANY IRISH ANCESTORS? THEN THIS STORY SHOWS WHY YOU'RE HERE.
Irish Potato Famine - 1840's
Warmer winter followed by a normal (mostly) warm growing season but late summer turned cool and rainy.
Leaves and stems seemed to "melt" away as they rotted and the potatoes were rotten and smelled WAY BAD!

Count DeBary saw white fuzz on leaves of sick plants which he knew meant a fungus was present, and wondered if there was a connection to the disease.

EXPERIMENT:Had potato plants in cold wet conditions
Half the plants had fungal spores,
half were kept from any spores.
Only those with spores got disease.
WHAT DOES THIS PROVE?

There are 3 CONDITIONS FOR A PLANT TO GET A DISEASE

-must have a PATHOGEN present
-must have proper ENVIRONMENTAL CONDITIONS
-host plant must be SUSCEPTIBLE (= can catch disease)
(Of 1000s of plant diseases 1 species of plant will only be susceptible to 10 to 20)

DO YOU SEE THE 3 CONDITIONS IN THIS STORY?
{Millerdent-Bourdeaux mixture, discourage poachers, copper shell casings in the 1840's)

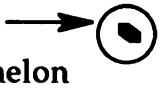
THE PATHOGENS

FUNGUS ARE THE WAY MOST IMPORTANT!!!!!!!!! 80% of Diseases

Examples - Rusts, smuts, Dutch elm disease, Chestnut blight, black bread molds, storage rots of fruits and vegetables, leaf spot, Black scurf (on potatoes)

BACTERIA are second

Examples - Lilac leaf spot, Soft rot (squishy potatoes in the bottom of the bag WHY? - the bacteria has made enzymes that digest pectin, freed sugars and other goodies are absorbed by the bacteria.

VIRUSES 

Example - Tobacco ring spot - you've seen it on watermelon and cantelope

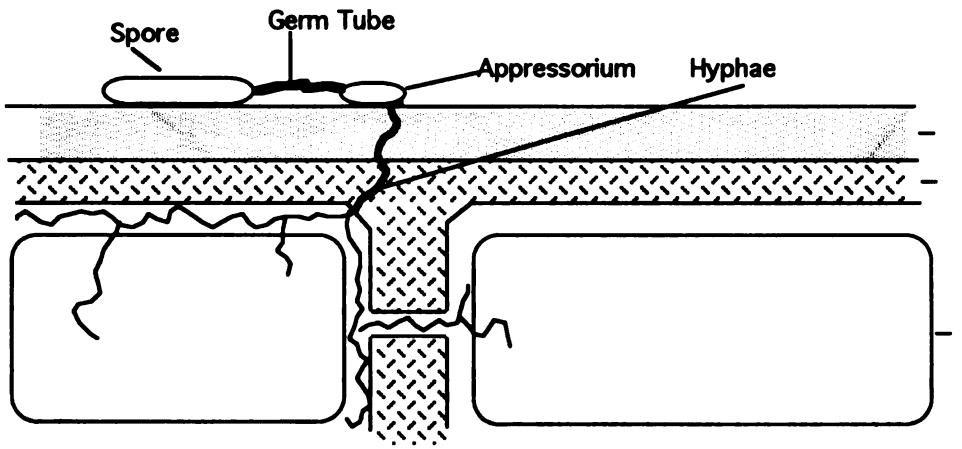
HOW PATHOGENS ATTACK AND PLANTS FIGHT BACK

FUNGI-

1)spore lands in good spot on outside of plant

2) spore grows a germ tube, an appressorium (glued on), then HYPHAE which begin eating it/their way into the plant

3) Usually plant doesn't react until hyphae reaches as far in as cell membrane



PLANT DEFENSES

Passive

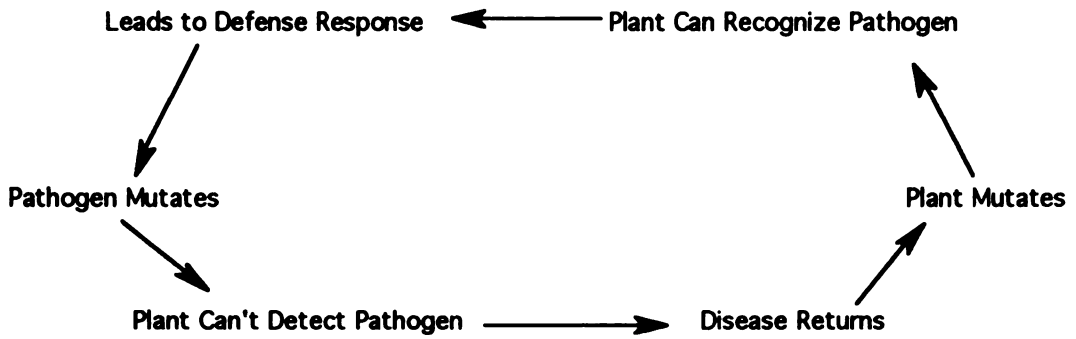
- •solid barriers
  - •hard surface (potato skin)
  - •wax and cuticle (very thick on stems or skin of green pepper)
- •chemicals
  - •phenolic acids (white onions get smudge, purple don't)
  - •toxic compounds (terpenes, the smell of pines like in Pine-Sol)

Active

- •quick, local cell death - fungus starves because next cell is already dead also stored poisons leak out protecting by killing fungus

- •immune response other cells detect death by chemical messages and produce other offensive and defensive chemicals
  - •chitinase and B-glucanase that breaks down fungal cell walls
  - •phytoalexins - are antibiotic and rapidly absorbed by fungi
  - •lignin- a very hard material plant uses to make a new wall keeping out pathogen, it is usually part of the xylem and other fibers

PLANT AND PATHOGEN PLAY AN EVER INCREASING GAME OF WEAPONS



What allows a plant to recognize a fungus is probably something on the hypha surface sort of like an antibody in people.

In plants the protective mutation lasts about 5 years so breeders are always looking for new resistant plants. If a fungus is not highly sexual the resistance will last longer.

Appendix G

PLANT PATHOLOGY SLIDES

25)BASIDIOMYCETE - bracket fungus, most of the hyphae (or body) is inside the log

24) LICHEN - a fungal/algal symbiont

23) SLIME MOLD - on shredded bark, plasmodium

7) LATE BLIGHT - dark half of leaf is water soaked and rotting because of infection, visible fuzziness is hyphae, other half appears to not be infected

8) LATE BLIGHT - on potato tuber, fuzzy parts are spore producing

6) LATE BLIGHT - killed tomatoes

1) RHIZOCTONIA - micrograph of hyphae, note divisions between cells and branching (possibly spore formation)

21) RHIZOCTONIA - on potato stems, lesions can girdle

2) RUST on cottonwood leaf, note spore pustules

3) CORN SMUT - note black spore producing structures

4) POWDERY MILDEW - on barley leaf, individual hyphae are visible, it is an obligate parasite

5) SCLEROTINA - it's a plant stem in X-section, the black lumps are sclerotia or hard-walled resting structures, is a pathogen to several plants

18) VERTICILLIUM causing WILT - vascular tissue blockage due to breakdown of xylem by enzymes and large number of spores produced, infects through roots to vascular tissue. Only some vines in this shot are infected.

26) COLLETOTRICHUM - on cucumber leaves, susceptible plant on LEFT, plant with some resistance on RIGHT, enters through stoma or leaf surface

CLADOSPORIUM - no pictures as it dissolves plant, rots younger tissue so it will work it's way down stem until it gets to and stops at tissue of a certain age

22) BLACK ROT - on Arabidopsis, in early stages, started at leaf tip, caused by the bacterium Xanthomona

9) PHYTOPHTHORA - on unripe pumpkin, white patches is sporulating fungus and probably where infection started, light green is rotting and infected, dark green maybe infected but not producing visible symptoms - yet!

19) PHYTOPHTHORA - micrograph of spores, note thick walls, flat bottom on left spore where stalk was attached

20) ALTERNARIA - micrograph of spores, note thick walls and multiple sections, see where stalk was attached

10) PSEUDOMONAS - bacteria on a petri dish

11) CLAVIBACTER causing RING ROT - a gram stain

12) ANGULAR LEAF SPOT - on cucumber leaf, bacterial disease (Psuedomonas) mostly follows veins causing necrotic lesions

13) CUCUMBER WILT - on cucumber leaf, bacteria live in and plug xylem, mainly gets in with the saliva of Cucumber Beetle so infection is carried from one plant to the next

14) XANTHOMONAS causing BLACK ROT - on cabbage leaf, is devastating to crucifers, enters through hydathodes (large openings at leaf margins directly hooked to vascular tissue) as root pressure overnight pushes water out and produces drops that are part of dew, bacteria naturally on leaf surface move into drops, as day heats up drops are drawn back in sucking bacteria with it. Bacteria colonizes xylem moving back progressively from the edge.

15) STREPTOMYCES - some are pathogenic to plants

16) STREPTOMYCES - filamentous growth form and divisions between cells is visible in this micrograph

17) STREPTOMYCES causing SCAB - on left has caused skin to produce cork like material
RHIZOCTONIA - on right, in a resting stage that is not a disease to the tuber but is waiting there to attack next springs vegetation

Appendix H

AMERICAN CHESTNUTS AND CHESTNUT BLIGHT
(Castanea dentata and Endothia parasitica)

The American Chestnut was an important tree. It was very common in forests of the Eastern U.S. One out of four trees in the Appalachians was a chestnut. The nuts were important as a food source - it was a major part of the diet for squirrels, raccoons and others. Along with oak, chestnut bark was the major source of tannin for the leather industry. The wood is as rot resistant as redwood and was used for fenceposts,railroad ties, furniture, etc. In Italy, a different species of chestnut is often used in place of Wolmanized lumber. Then there is the empty place in American history and culture:

Under the spreading Chestnut tree,
The village smithy stands - Longfellow
OR
Chestnuts roasting on an open fire
Jack frost nipping at your nose.

This all changed beginning in 1904.

1904-Strange illness noticed on trees at New York Zoological Gardens.

1906-A new fungus then named Chestnut Blight was positively identified as the cause of the disease

1908-All states in Northeast U.S. report blight is widespread.

1912-Blight widely reported down to Georgia and out to Iowa and Nebraska.

1913-Blight is found on different species of chestnut in China and Japan but the trees are not affected at all.

1925-Illinois reports that all of it's chestnuts are dead.

1929-Blight reported in Washington and Oregon.

1938-Blight is found and is damaging chestnuts of yet another variety (Castanea sativa)in France and Italy.

Considering that the first appearance of blight in the US and Europe was at a port city and that the fungus doesn't harm the Asian trees probably means that humans carried the fungus here. In other words, all the damage caused

by Chestnut Blight economic, environmental and etc is something we could have prevented because we didn't know any better. Ahh, the dangers of technology. Score one more bright move for us.

AND NOW..... MORE ABOUT THE FUNGUS

-Spores enter the plant through wounds, germinate in summer and grow fast.

-The hyphae lives between cells, sucking food much like a vampire.

-It doesn't directly try to kill the cell but damage occurs as the mycelium grow so large it crushes and blocks plant tissue, especially the vascular ("veins") tissue.

-The fungus can also eat on the dead chestnut wood, if it has to.

-Can live in any part of the plant but likes the inner bark best. When it grows here it forms an ugly, lumpy mass called a canker.

-Since there is vascular tissue in the inner bark the "veins" get blocked.

- If the canker goes all the way around a tree then the tree has been girdled and the parts above will eventually wilt and die.

-Orange or red spots on the cankers are structures that shoot out spores to be carried by wind or animals to another tree.

RESTORING THE CHESTNUT TREE-just a dream?

-There has been some work on cross-breeding the smaller Asian chestnut species with the American but the trees are not as resistant as the Asian species and don't get very tall.

-In Italy, 1951, it was noticed that some chestnut trees had the Blight but were not dying or even being hurt too bad - this variety of the fungus was called hypovirulent (or "less deadly" or hey let's cut to the chase - weaker!)

-There seem to be lots of different types of hypovirulent Blight fungus but that all of them have a virus - so.....the disease has a disease!

-If you grow a hypovirulent fungus touching a killer then the virus will be transferred. So it seems like all you'd have to do is put the hypovirulent fungus out there and all the killer fungi would get sick and not kill Chestnuts

-BUT the hypovirulence doesn't seem to move to another tree all by itself so unless we spend BIG$$$ and pay people to find and treat every chestnut tree this won't bring them back BUT we can do cool things with Chestnut Blight Fungus in the classroom.

Appendix I

TISSUE CULTURE NOTES

-**Plant tissue culture** is the production from 1 plant of many genetically identical plants called clones using hormones.
{The traditional method is to take one large plant and divide it into 2 or 3 parts which are then planted in the ground and allowed to grow up for 1 to 3 years.}

It is useful to plant breeders because
-larger numbers (than w/ traditional methods) of plants can be produced from a single plant
-virus-free plants can be raised,
-the second generation of plants is usually more vigorous
-speeds up crop improvement and plants can be genetically tailored for certain conditions.

If tissue culture could be done with animals like it is with plants then we might have a real "Jurassic Park".

3 Stages
Germination
-need young, healthy plant tissue as a source of cells
our source is radish and carrot seedlings in a "sterile" environment
-germinated on disposable petri dish containing germination media (Phase 1) after seeds have been soaked in sterilizing Silver Nitrate
Stimulating Callus Tissue
-Phase 2 media causes cells to grow into undifferentiated mass (glob) of cells called callus tissue with plant hormones
-**undifferentiated** means the cells have a shape not suited or specialized for a particular job
Stimulating Whole Plant Growth
-after callus has formed, the hormones in the Phase 3 media will cause the growth of roots and stems and eventually a whole plant

-a **HORMONE** is a body chemical. In animals they are produced in glands and travel through the blood to the whole body but only certain body parts (where they are absorbed into that parts' cells) respond to their message. In plants they travel through the sap and diffuse into cells. Cells that react to the hormones message have a specially shaped receptor and when the "LOCK" meets the "KEY" the cell responds by doing something that involves chemicals (because we are all basically chemicals).
SOOOOO.....-they are not directly involved in chemical reactions
-they usually cause a whole series of things to happen

Appendix J

TALES FROM THE PETRI DISH FINAL EXAM - WINTER SEMESTER '96'

NAME_____

1) In microbiology, the purpose of sterile technique is:

2) List 3 ways to sterilize using heat:
   1.
   2.
   3.

3) List 2 common liquids used for sterilizing (including the percentage of the liquid usually most effective):
   1.
   2.

4) "Invert", means to:

5) Why would a petri dish be "inverted"?

6) Describe the proper way to remove a petri dish lid to work on it:
   1.
   2.

7) List the 4 steps in transferring a bacteria or other culture from one petri dish to another:
   1.
   2.
   3.
   4.

8) List and briefly discuss 2 ways not mentioned above, to reduce exposure of a person or culture. I don't have a specific answer in mind. Use common sense.
   1.
   2.

9) Define the word "media" as used in microbiology

---

10)Define MICROBIOLOGY in your own words. Also, list as many jobs as you can think of that use microbiology knowledge or skills. You can use the back of the page.

11) An enzyme: (CIRCLE ALL THAT APPLY)

(a) is used up in the processes they are part of
(b) is directly involved in chemical reactions
(c) usually stimulates cells into doing something with chemicals.
(d) is a nutrient or source of food
(e) is not used up in the processes they are part of
(f) can cause more than 1 action to happen
(g) is not a nutrient or source of food
(h) will cause 1 and only 1 action to happen.

12) T or F An enzyme is a type of carbohydrate.

13) T or F Enzymes were originally found in living things

14) T or F Enzymes can work inside and outside of organisms

15)The speed that an enzyme works at depends on: (CIRCLE ALL THAT APPLY)

(a) concentration of the enzyme
(b) amount of available light
(c) presence of inhibitor(s)
(d) pH
(e) radiation
(f) temperature
(g) amount of available protein

16) The names of enzymes usually end in_____

17) The names of sugars usually end in_____

18) The weight of bacteria living in the average human body is about equal to

(a) a gram
(b) nothing, none live in our bodies
(c) seven pounds
(d) the weight of a 12 ounce can of pop.

19) DISCUSS this statement. "Bacteria only live in special locations."

---

20) The first forms of life on Earth appeared about 3 billion years ago & were probably

a) bacteria b) fungi c) viruses d) plants

21) Bacteria and fungi do not have mouths, how does their food get in?

22) Complete this chart about the differences and similarities between viruses, bacteria and fungi. **Words in parentheses ( ) are choices to use as answers, but those in brackets [ ] are only for explanation.**

	FUNGI	VIRUSES	BACTERIA
"cell" organization (single, colony, multicellular)			
method of getting food [or new "cell" material]			
internal membranes (yes or no)			
exterior mat'l (chitin, protein, protein/lipid)			
rank in size (smallest, medium, largest)			
what gas is required to live (oxygen, other, and none)			
style of reproduction (sexual,asexual, neither)			
alive or not (yes or no)			

23) About 5,000 to 10,000 species of bacteria have been discovered and named. This is only about _____ percent of all the species of bacteria that probably exist.

24) Most scientists who study them agree that it easier and more useful to identify bacteria by their abilities than by their appearance. TRUE FALSE

25) Yeast is an example of what type of organism?_____

26) The 3 shapes of single bacteria cells are: (Draw a small, simple picture of the shape also.)

a.

b.

c.

27) Single bacterial cells group together in what three ways:
a. b.

c.

28) Of the 6 following words there are 2 groups of 3 that make sense together. Put them in the right groups on the lines below.
aerobic, anaerobic, with oxygen, without oxygen, respiration, fermentation

Group 1_____

Group 2_____

29) How many bacteria does it take to start a colony of bacteria on a petri dish media ?_____

30) The purpose of selective media is to find what concentration of something (pH, salt, etc) a bacteria (or other micro-organism) likes best. TRUE FALSE

31) The purpose of a serial dilution is: (best answer only)

(a) to keep unwanted organisms from growing
(b) to find what concentration of something that bacteria like best
(c) to find the best concentration of something depending on the purpose of the dilution

32) In a serial dilution if the concentration of the first container is 10:10 and the concentration of the second container is 9:10,, then list the concentrations of the next 4 containers (extra credit -list % of desired substance in the solution).

CONTAINER	1 & 2	3	4	5	6
CONCENTRATION	10:10&9:10				
PERCENTAGE					

33) Give one example of something serial dilution might be used to find out.

---

34) The purpose of both fermentation and respiration for micro-organisms is

---

35) TRUE or FALSE Most foods produced using microbes involve fermentation.

36)TRUE or FALSE Most beverages produced using microbes involve fermentation.

37) TRUE or FALSE Fermentation always results in the production of alcohol.

38) When a micro-organism is used to produce a food or beverage,

it "eats" _____ using _____ and then

makes_____

39) Lactobacilli use _____ to digest _____ and are used by the food industry to make _____and_____.

40) All production of beer and wine depends on what microbe?_____

41) Name some of the most commonly desired by-products of microbial activity

1_____2_____3_____

42) Choose one of the phrases below. Describe **which micro-organisms** are **doing what with what** and how that **results** in the desired product.
a) Hops, Malted Barley & Water into Beer
b) Granulated Sugar, Lemon, Ginger, Tartar into Ginger Ale

43)Define the following terms-

plant pathology

pathogen.

hypovirulent

virulent

44) List the three major groups of organisms that cause plant diseases in order from most diseases to least diseases.

45) {Explain why} When we cultured Chestnut Blight fungus from bark we
a) soaked the bark in bleach

b) but for only 5 minutes.

46) If Potato Rot fungus was around in a potato field and the weather was hot and dry, would a large number of the potatoes probably get the disease? Explain your answer.

47) What one development in microbiology has significantly increased the human lifespan since World War II?

48) Explain what is happening in the diagram shown below.

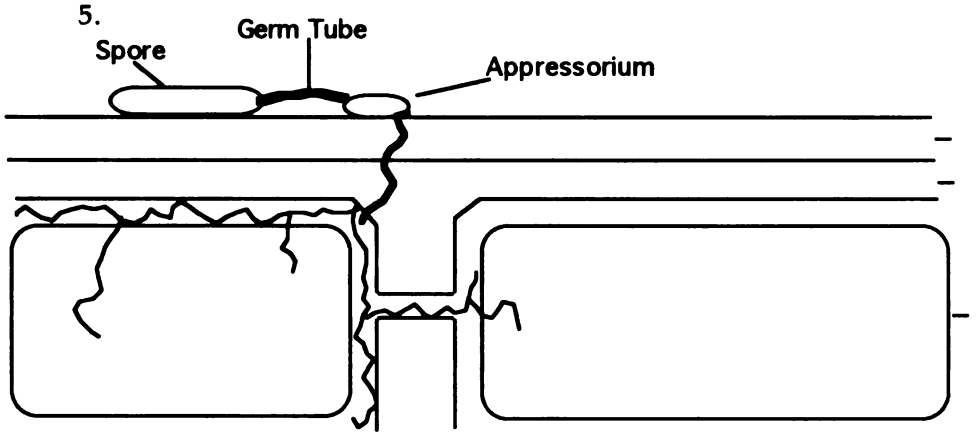
1.

2.

3.

4.

5.



49) We will culture Chestnut Blight from bark and grow it on a PDA dish **with a different type of Blight.** Predict what you think will happen.

50) Talk about the questions below.
a) Why was the American Chestnut an important tree?

b) What happened to it? (when, how long, from where, how many)

c)Why is the chestnuts' decline important for ourselves and society to think about?

51) There are 3 conditions for a plant to get a disease. The third condition is that the plant is susceptible.
(a)What does susceptible mean

(b) Why is it worth mentioning especially when we are discussing plant diseases?

52) What is the difference between passive and active plant defenses?

What sort of defenses to disease do plants have?List and explain some.

53) We will infect a cucumber plant with a weak plant disease, then a few days later with a second stronger disease. Describe what will happen to the plants resistance.

54) A plants ability to resist a pathogen lasts for only a few years and vice-versa. Explain why this might be.

55) List as many things as you can think of that would happen in a Medical Technology Lab like one found in a hospital.

Appendix K

STUDENT PRE-CLASS INTERVIEW

1) What Is Your:
Name
Age
Sex
Born Where,
Living Where

2) What is name of this class?

3) What have you heard about the class before this moment?

4) Why did you choose to take this class?

5) What do you expect to do in the class?

6) Do you expect to be excited by or (dis-, just plain, or especially) interested in the following parts of the class?
- Experiments
- Field Trips
- Information

7a) This is a class about microbiology, does that tell you something new or different about it?

7b) What is microbiology or is usually considered to be part of it?

8) What is a microbe?
- List types
- List characteristics

9) Who uses microbiology and how.
- In General
- In making or studying foods
- In studying plant diseases

10) What ideas do you expect to learn from this class?

11) What skills do you expect to learn from this class?

12) Do you think there are any skills or information from this class that you will be able to use in:

the real world,

a future job

a later science class

13) Do you think it is valuable to simply have general knowledge (such as how cheese is made) that you may not use in the real world, a future job or a later science class?

14) Have you considered a career in science or a related field?

15) How do you feel about science classes in general (interested, intimidated, had 1 good past teacher, etc)?

STUDENT POST-CLASS INTERVIEW

1) What Is Your:
Name
Age
Sex
Born Where,
Living Where

2) What is name of this class?

3) Was the class what you expected it to be?

4) Are you glad that you chose this class or do you wish you had taken another?

5) List 3-5 things you did in this class.

6) Were you excited and/or (dis-, just plain, or especially) interested in the following parts of the class?

Experiments
Field Trips
Information

7) What is microbiology or is usually considered to be part of it?

8) What is a microbe?

List types

List characteristics

9) Who uses microbiology and how.

In General

In making or studying foods

In studying plant diseases

10) What ideas did you learn from this class and/or How has taking this class changed your view of the world?

11)Name 3-5 skills you have learned from this class.

12) Did you learn any skills or information from this class that you will be able to use in:

the real world,

a future job

a later science class

13) Do you think it is valuable to simply have general knowledge (such as how cheese is made) that you may not use in the real world, a future job or a later science class?

14) Now that you have taken this class would you consider a career in science or a related field?

15) In the pre-interview you said ____ about science classes in general, has this class changed your feelings about that or how do you feel about this class compared to others you've taken?

Appendix L

FIELD TRIP TO WINERY REPORT FORM

1) WHAT IS THE PURPOSE OF THIS FIELD TRIP?

2) WHAT ARE SOME NUMBERS THAT DESCRIBE THEIR BUSINESS?

3) COMPARE THE IMPORTANCE OF STERILE CONDITIONS AT CAMPBELLS AGAINST THE WINERY.

4) WHAT WAS THE BEST PART OF THE TRIP? WHAT WAS THE WORST PART OF THE TRIP (not including the bus ride)?

5) WHAT CARBONATED BEVERAGES DO THEY PRODUCE AT THE WINERY?

6) DIAGRAM THE PROCESS OF PRODUCING WINE AT ST. JULIAN (Use back of paper).

Appendix M

TISSUE CULTURE EXPERIMENT

Plant tissue culture is production of many identical plants or clones from 1 plant. It is useful to plant breeders because virus-free plants can be raised, speeds up crop improvement and plants can be genetically tailored for certain conditions. If tissue culture could be done with animals like it is with plants then we might have a real "Jurassic Park".

Seedling pieces will be placed on one of four different medias. The media are Murashige and Skoog A, B, C and Control. The A, B and C media vary in their proportion of the hormones kinetin and indole acetic acid. The Control media contains neither hormone.

3 Stages
Germination
-need young, healthy plant tissue as a source of cells
our source is radish seedlings in a "sterile" environment
-germinated in autoclaved petri dish and paper towels, moistened with autoclaved bleach solution (3:1, water:bleach)

Stimulating Callus Tissue
-cause cells to grow into undifferentiated mass (glob) of cells called callus tissue with plant hormones
-cut sections of stem are placed on media containing kinetin &/or IAA, the stems absorb the hormones from the media
-undifferentiated means the cells have a shape not suited or specialized for a particular job
-see your Tissue Culture Notes to be refreshed as to what a hormone is

Stimulating Whole Plant Growth
-after callus has formed the hormones will cause the growth of roots and stems and eventually a whole plant

MATERIALS

Alcohol	Wax Pencil
5% Bleach Solution	Radish Seedlings
Tweezers	Sterile Petri Dishes, 250ml Beakers
Alcohol Burner	Autoclave (pressure cooker) and Oven
Enclosed Work Area	Murashige & Skoog tissue culture media- A,B,C & Control

PROCEDURE
Wash hands with soap and water.
Sterilize work surface
Media Preparation
1) In 2x container add 90% of final volume water
2) While stirring, add powdered media.
3) Rinse media container into 2x container with small volume of water.
4) Add agar.
5) While stirring adjust to pH 5.7 + or - 0.1.
5) Bring to final liquid volume.
6) Heat solution to 100°C while stirring.
7) Autoclave media. Label plates appropriately - Control, Media A, etc.
8) Pour plates. Allow to solidify at room temperature

Placing Seedlings On Media
-Inside Enclosed Work Area(glass aquariums):
-Set up work area: sterilize glass inner surfaces with bleach, the empty sterile petri dish bottom will be where you cut up seedlings, the top will serve as the cover for the 250ml beaker. The alcohol lamp will be burning outside.
-Top layers of moist paper towel can be removed from seed dishes.
-Remove 1 germinated seedling from dish with sterile tweezers, re-cover dish.
-Inside a second sterile dish and with a sterile razor blade cut off all root and shoot tissue. Cut remaining stem into sections between 1/2 and 1 cm. long.
-Flame razor in-between each seedling.
-With sterile forceps put stem sections into sterile beaker, cover with other half of sterile dish.
-After all seedlings are cut up, add 70% alcohol, cover and shake gently for exactly 1 minute.
-Pour off alcohol (using lid to keep stems from falling out) then add enough 10% bleach solution to cover, put lid on and shake gently for 5 minutes.
-Pour off bleach, then rinse stems 3 times with sterile distilled water.
-With sterile forceps put stem sections onto media. 4 sections of stem can be evenly spaced around the petri dish.
-Tape dish closed and put it in clear plastic bag.
-Leave bags in location with appropriate light and temperature.
Temp 74-80°F constant, especially during regeneration stage.
Light from window is adequate, incubator is preferred.
Keep cultures in clear plastic bag (to reduce airflow).

Follow-up
-Remove contaminated cultures to another bag when discovered.
-Cell division should begin to produce a callus shortly (1 week ?). It should be at an ideal size of about 1 cm. diameter in 1 month or so. Shoots and roots should begin to appear shortly.

Appendix N

BREWING BEER

Fermentation is an important chemical process for the baking and brewing industry. Beer is made as the result of a fermentation and involves four important ingredients.

Fermentable sugars (usually malted barley)
Hops
Water
Yeast

1) Malted barley is produced naturally by putting barley seeds into water where they germinate. This means enzymes are breaking down stored starches into sugars, a process called mashing. After the process is done and the seeds are dried they are now said to be malted. It is these sugars that the yeast will ferment into alcohol, carbon dioxide and the flavor of beer. Sometimes breweries replace some of the malted barley with malted corn, rice, wheat, rye, or another grain.

There are two kinds of enzymes doing different but important jobs in mashing. Proteases are breaking down proteins into amino acids. The yeast uses these to build/grow more yeast cells. This process helps to improve the clarity and foam potential of beer. Alpha-amylase and beta-amylase break down the starch which is a bunch of very long chains of glucose molecules. Alpha-amylase cuts the long chains in the middle, repeatedly, until the chains are quite a bit shorter. At the same time the beta-amylase works by "nibbling" at the ends (an enzyme can't really nibble because it's not alive). Beta-amylase cuts off chains that are one, two or three glucose molecules long. At this size they are fermentable sugars.

2) Hops are green cone-shaped flowers that grow on vines and have been used in brewing for thousands of years. Hops give a bit of bitterness to beer that balances the sweetness of the malt. They also retard spoilage and add to the head of the beer.

3) Beer is 90% water. The water provides the proper environment for yeast to ferment the sugars of the malted barley.

4) Yeasts are a type of fungi and are living microbes. Thousands of different kinds can be found. Most of the yeasts used in brewing are called BREWER'S YEAST (Usually *Saccharomyces cerevisiae* for ale and *Saccharomyces carlsbergensis* for lager). Most brewers guard the identity of

the specific species of yeast that they use. Baker's yeast is used for making bread.

CLEANING BOTTLES

1) Wash bottles out in hot water and detergent.
2) Rinse out soap
3) Sterilize by immersing in 10% bleach solution for 5 - 10 minutes.
4) Rinse with clear water until bleach odor has disappeared.
5) Drain the bottles dry.

PREPARING FERMENTATION LOCK

As the yeast ferment the sugar they will produce a large quantity of carbon dioxide gas. At the same time we want to prevent contamination by air-borne microbes and keep oxygen from entering the bottles. Both needs can be met with one piece of equipment. Invert a gallon jar of water in a pan of water. The jar should be secured so that when it is full of gas it does not tip and the jar should also have a spacer underneath so that the hoses from the bottles are not crimped. Also, be ready to manage the water level as the yeast will produce more than enough gas to empty the gallon jar.

BREWING INSTRUCTIONS

1) However many bottles are being brewed determine the size of the master mix. Mix 55 grams of malt extract and 0.28 g. of hops in 350 ml of tap water for each bottle of beer. Put all of this in one large pot.

2) Bring to a boil and boil for 5 min.

3) Strain the mixture from step 2 (it's called WORT) through cheesecloth into a 1L flask and cool to 70 degrees F

4) Find the beginning alcohol content by pouring a small amount into the hydrometer vessel, then slowly lower the hydrometer into the liquid (don't

spill). Record the specific gravity shown on the hydrometer_____ then pour this sample down the drain.

5) Pipet 7 ml of JOEY D'S YEAST SOLUTION into a clean cappable bottle. Add enough wort to bring the liquid up to just past the shoulder of the bottle.

6) While the bottles are being filled, take a drop of the mixture from the beaker and make a wet mount slide. Count the yeast cells on the slide using medium power in each of the 4 corner areas and the center and the find the average. This is the initial population at TIME 0. Each day for the next several we will CAREFULLY open one or two of the classes bottles and make the same type of count. Record this information each day below.

7) Insert a one-hole stopper with a length of tubing attached and insert the tubing under the inverted jar to form a fermentation lock to allow fermentation to begin at room temperature.

8) After vigorous bubbling subsides (1 - 2 days) remove the fermentation lock and cap the bottle.

9) Allow beer to mature at room temperature for another 2 - 5 days.

10) Take a second hydrometer reading here_____. Do the math with this result and your first reading as shown in question #1

1)Beginning Specific Gravity _____

Final Specific Gravity _____

_____ X 105 = _____ % alcohol by weight

Alcoholic Proof _____ % alcohol by weight X 2 = _____Proof

Extra credit: Do the same math but for some other day or days before the final day and do it on a separate sheet of paper.

2) Why are water, malt extract and hops boiled in step 1 before adding yeast?

3) Why take a hydrometer reading before adding yeast and after fermenting?

4) What is the purpose of the fermentation lock and how does it work?

<u>Yeast population Data Analysis</u>

1) Graph the average yeast population versus time.
2) Explain the shape of this curve.

Appendix O

CHESTNUT BLIGHT

Isolating The Fungus, Transmitting Hypovirulence, Testing For Transmission

Background

The word virulent (V) means that a disease causing agent is strong or as in the case of Chestnut Blight, that the fungus can kill a tree. Hypovirulent (H) means the disease agent is weaker and would probably not kill a tree though the tree may look messed up. On PDA it is often possible to visually tell if a mycelium is H or V. A V fungus will have pretty much smooth and round edges and there will be concentric rings of various colors. The edges of an H fungus will be deeply lobed and the colors won't be as neatly separated.

Materials

Plates of PDA agar
40% Bleach Solution
Small containers to soak bark
Sterile Water
Sterile Paper Towel

ISOLATING THE FUNGUS

1) Collect bark from an American Chestnut in the area immediately around a canker.

2) Put a piece of bark in 20% bleach solution for ~5 min. Make all further transfers using sterile technique.

3) Rinse bark twice by soaking in new sterile water for 1 min. each time.

4) Dab dry with a paper towel.

5) Transfer bark to PDA plates and label appropriately. More than 1 piece of bark may be on a plate though the colonies may grow overlapping.

6) Incubate at room temp. for 5 or more days, observing the plates daily. When mycelia develops, decide by appearance if fungus is H or V.

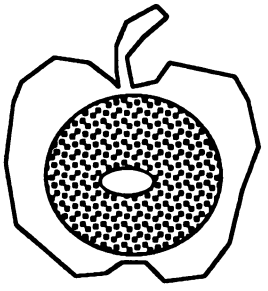
TESTING FOR HYPOVIRULENCE

1)Remove a 1/4" to 1/2" deep plug (about the size of 1 to pencil erasers) from a Golden Delicious apple.

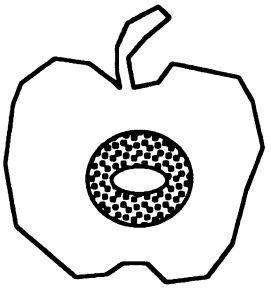
2) Put a chunk of whichever fungal cultures are being tested into the hole. Pack in so that all the inside of the hole is touching fungus. Replace some of the apple and scotch tape the hole closed to reduce contamination.

3) Allow 14 or 21 days for decay, then inspect and decide by the size of decayed areas as shown on the diagrams below, if the fungus is H or V.

V≥
60-80mm



H≤
~30mm



EDGE OF ROTTEN AREA

Chestnut Blight Fungus
-a soft brown color
-firm edge that's very circular
-apple meat would core out like a melon ball

Other Fungi
-light or yellow brown
-irregular, squishy
-wouldn't core out

TRANSMITTING HYPOVIRULENCE

1) Transfer a small section of the newly isolated fungal colonies to a new PDA plate.

2a) If suspected to be V, also add a small piece of the fungus E17f.

2b) If suspected to be H, also add a small section of the fungus Magnificent 7. The transferred pieces should be an inch or two apart and towards one side. That way as the mycelia grows you will be able to observe that it has the expected appearance at the beginning and there will also be room for the anastomosed mycelia to clearly show an altered appearance. Incubate at room temperature for 5 more days. Observe each day.

3) If the hypovirulence was transferred then at the point where the two mycelia meet there should begin to be a change in the appearance of the V fungus, looking more like an H type.

NOTE: 3 rules for determining if a fungus is truly H it must:
- -be less virulent than normal
- - be able to be transfer it's hypovirulence by hyphal anastomosis
- -be caused by cytoplasmic factors such as virus

Appendix P

ACQUIRED SYSTEMIC RESISTANCE IN CUCUMBERS

Objective: Demonstrate that some plants can be induced by non-lethal attack of one pathogen to resist a second attack by a second, different pathogen.

Materials:
Pathogen Cultures - Colletotrichum lagenarium (Anthracnose Fungus)
produces necrotic lesions on leaves
- Cladosporium cucumerium (Scab Fungus)
kills entire plant
Cucumber Varieties - SMR 58 - genetically resistant to Scab Fungus,
susceptible to Anthracnose Fungus
- National Pickling - susceptible to both Fungi
Distilled water
Glass "hockey stick" or stirring rod
Pipette
Small flask or beaker

Procedure:
1) Germinate then plant cucumber seeds 2-3 weeks prior to use. Should have 2 sets of true leaves at time of first inoculation.

2) Culture pathogens for 7-10 days prior on PDA (Potato Dextrose Agar) or on V-8 agar.

3) When spores have developed, add a few mls. of distilled water. Rub vigorously with glass rod but don't tear media. After a good rub, pour the liquid (this is the spore suspension) into clean container.

4) Put 0.1ml drop of spore suspension on slide, put on a cover slip and count spores. The desired amount is around 5 X 10^5 spores/ml and there are 1000 fields across a cover slip, so under medium power in 5 fields (top, bottom, left side, right side, center) you should see an average of about 500 spores/field.

5) TO INDUCE RESISTANCE
Depending upon which of the comparisons (shown below) you are investigating, inoculate the first true set of leaves on each plant with a 5-10 microliter droplet (30 droplets/leaf) of the appropriate spore suspension. Incubate in a humidity chamber (can be a plastic bag or box, anything where the humidity can be maintained at 100%) for 24 hours.

THE COMPARISONS

A) To see if the inoculation with the first fungus actually does increase resistance to a second fungus.

First Inoculation Second Inoculation

Distilled Water Only VS. Spore Suspension

B) To see how much protection an inoculation provides from a 2d inoculation with the same fungus.

Scab VS. Scab and Anthracnose VS. Anthracnose

C) To see if the first fungus must be a weaker species to provide resistance to a stronger one.

Scab - first, Anthracnose - 2d VS. Anthracnose - first, Scab - 2d

6) CHALLENGE INOCULATION: Open incubation chamber and allow to equilibrate with environment. Inoculate second set of true leaves as described above with the second spore suspension at the same concentration. Asses over 2-4 days the condition of the challenged leaves and the plant as a whole.

TASKS

1 Figure out how many cucumber seedlings should receive each type of the first inoculation?

2 Of these, which ones should be in the same humidity chambers?

3 Figure out how many cucumber seedlings should receive each type of the second inoculation?

4 How can we measure the difference in resistance?

5 Look at the comparisons. Predict outcomes for the three experiments and say what you would see or measure when looking at the plants.

Appendix Q

PD3, 4 and 5 Student Pre-Test and Post-Test Results

Student Number	Pre-Test		Post-Test	
	Number Correct	Percentage	Number Correct	Percentage
1	26/100	26	77.5/100	77.5
2	19/100	19	80/100	80
3	33/100	33	95/100	95
4	26/100	26	42/100	42
5	27/100	27	83/100	83
6	34.5/105 52/104	32 50	75.5/110 75/104	68 72
7	27.5/105	26	70/110	63
8	28.5/105	31	78/110	70
9	22.5/105	21	72/110	65
10	28/105	26	84/110	76
11	21/105 49/104	20 47	60/110 68/104	54 65
12	26.5/105	25	36/110	32
13	51/105	48	106.5/110	96
14	27/105	25	76/110	69
15	33.5/104	32	97/104	93
16	23/104	22	67.5/104	65
17	34/104	33	87/104	84
18	25/104	24	80.5/104	77
19	20.5/104	18	53.5/104	51
20	17/104	16	32/104	30
21	26/104	25	77/104	74

22	19.5/104	19	59/104	56
23	26/104	25	60/104	58
24	28/104	27	57.5/104	55
25	27/104	26	56/104	54
26	22/104	21	45.5/104	43
27	15/104	14	80.5/104	77

Appendix R

PD3, 4 and 5 Student Demographic Data - Age and GradePoint

Student Number	Date Of Birth	Age At Start Of Class	G.P.A. Prior to BAHS	G.P.A. Prior to PD class	G.P.A. During PD class
1	3-1-79	15	1.80(8th)	3.30	2.67
2	9-1-78	16	NA	1.63	1.87
3	11-16-76	17	2.40	3.00	3.17
4	1-3-79	15	0.00(7th)	1.75	1.00
5	10-10-79	15	2.57	2.45	3.32
6	2-8-78	16 17	0.80(7th)	3.30 2.90	3.42 2.08
7	4-27-76	18	1.00	2.45	3.10
8	8-29-76	18	2.72	----	3.25
9	4-8-77	17	1.80	3.83	2.67
10	1-19-77	18	1.50	3.15	3.50
11	8-26-78	16 17	2.50 ----	3.67 3.42	3.00 3.30
12	7-20-77	17	2.70	2.80	1.13
13	7-28-65	29	NA	3.80	3.58
14	2-12-78	16	0.00(8th)	3.10	2.36
15	10-25-80	15	1.11(7th)	3.00	3.67
16	6-20-78	17	1.80	3.17	2.00
17	2-25-80	15	1.1(8th)	2.90	3.30
18	1-27-78	17	1.71(Acad) 3.00(VoTech)	2.40	2.08
19	1-4-80	16	1.3	2.00	2.18
20	4-3-78	17	0.60	2.30	0.50

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16	6-20-78	17	1.80	3.17	2.00
17	2-25-80	15	1.1(8th)	2.90	3.30
18	1-27-78	17	1.71(Acad) 3.00(VoTech)	2.40	2.08
19	1-4-80	16	1.3	2.00	2.18
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4	1-3-79	15	0.00(7th)	1.75	1.00
5	10-10-79	15	2.57	2.45	3.32
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7	4-27-76	18	1.00	2.45	3.10
8	8-29-76	18	2.72	----	3.25
9	4-8-77	17	1.80	3.83	2.67
10	1-19-77	18	1.50	3.15	3.50
11	8-26-78	16 17	2.50 ----	3.67 3.42	3.00 3.30
12	7-20-77	17	2.70	2.80	1.13
13	7-28-65	29	NA	3.80	3.58
14	2-12-78	16	0.00(8th)	3.10	2.36
15	10-25-80	15	1.11(7th)	3.00	3.67
16	6-20-78	17	1.80	3.17	2.00
17	2-25-80	15	1.1(8th)	2.90	3.30
18	1-27-78	17	1.71(Acad) 3.00(VoTech)	2.40	2.08
19	1-4-80	16	1.3	2.00	2.18
20	4-3-78	17	0.60	2.30	0.50

21	5-2-79	16	0.8(8th)	3.20	3.08
22	1-14-79	17	2.80	----	2.40
23	3-12-79	16	0.67	3.50	3.50
24	12-8-77	18	2.00	2.83	1.25
25	9-4-78	17	0.50(Acad) 2.90(VoTech)	1.20	2.50
26	3-4-75	20	NA	2.18	3.00
27	1-10-76	20	2.0	3.40	2.75

Students 1 - 5 were in PD3, students 6 - 14 were in PD4 and students 15 - 27 were in PD5. Students #6 and 11 were in both PD4 and PD5.

Items in ( ): (7th) or (8th) indicate student did not previously attend high school. Some of the students with parentheses are older than their class but were retained in one of the upper grades.

Students 18 and 25 attended the Van Buren Vocational And Technical Training Center prior to BAHS. The (Acad) refers to the students GPA at their home school.

Appendix S

PD3, 4 and 5 Student Demographic Data - Attendance

Student Number	Block Periods Of Class Pres./Abs. - %	Percentage Of Days For Year	BAHS Entry Date	Grade On Entry
1	34/44 - 77	Unavail.	8-25-93	9
2	44/44 - 100	Unavail.	12-1-93	9
3	32/44 - 73	Unavail.	3-15-94	10
4	33/44 - 75	Unavail.	8-24-93	9
5	42/44 - 95	Unavail.	2-22-94	9
6	32/39 - 82 69/81 - 85	Unavail. 85	8-25-92 --------	9 -
7	35/39 - 90	Unavail.	8-24-93	10
8	30/39 - 77	Unavail.	1-23-96	11
9	28/39 - 72	Unavail.	1-17-94	11
10	29/39 - 74	Unavail.	2-21-94	10
11	38/39 - 97 80/81 -99	Unavail. 90	11-1-93 ---------	9 -
12	19/39 - 48	Unavail.	12-7-93	10
13	29/39 - 74	Unavail.	8-26-92	11
14	38/39 - 97	Unavail.	8-24-93	9
15	76/81 - 94	93	8-28-95	9
16	67/81 - 83	87	11-6-95	11
17	69/81 - 85	88	8-29-94	9
18	63/81 - 78	62	3-15-94	10
19	54/81 - 67	71	8-24-95	9
20	51/81 - 63	63	8-18-94	9
21	65/81 - 80	85	10-5-94	9

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1	34/44 - 77	Unavail.	8-25-93	9
2	44/44 - 100	Unavail.	12-1-93	9
3	32/44 - 73	Unavail.	3-15-94	10
4	33/44 - 75	Unavail.	8-24-93	9
5	42/44 - 95	Unavail.	2-22-94	9
6	32/39 - 82 69/81 - 85	Unavail. 85	8-25-92 --------	9 -
7	35/39 - 90	Unavail.	8-24-93	10
8	30/39 - 77	Unavail.	1-23-96	11
9	28/39 - 72	Unavail.	1-17-94	11
10	29/39 - 74	Unavail.	2-21-94	10
11	38/39 - 97 80/81 -99	Unavail. 90	11-1-93 ---------	9 -
12	19/39 - 48	Unavail.	12-7-93	10
13	29/39 - 74	Unavail.	8-26-92	11
14	38/39 - 97	Unavail.	8-24-93	9
15	76/81 - 94	93	8-28-95	9
16	67/81 - 83	87	11-6-95	11
17	69/81 - 85	88	8-29-94	9
18	63/81 - 78	62	3-15-94	10
19	54/81 - 67	71	8-24-95	9
20	51/81 - 63	63	8-18-94	9
21	65/81 - 80	85	10-5-94	9

22	57/81 - 70	86	1-22-96	11
23	75/81 - 93	90	9-5-95	9
24	63/81 - 78	87	1-19-95	10
25	56/75 - 69	72	1-29-96	11
26	46/81 - 57	78	1-22-96	12
27	51/81 - 63	72	8-25-95	11

Appendix T

PD3, 4 and 5 Student Demographic Data - Grade Level

Student Number	MEAP	CAT	Brigance	Iowa
1	Low	6.8		
	Low	6.9	---------	------
	10-94	3-94		
2	Low	7.4		
	Low	5.1	---------	------
	10-94	3-94		
3	Moderate	10.7		
	Moderate	10.3	---------	------
	10-94	3-94		
4	Low	2.3		
	Low	3.3	---------	------
	10-94	3-94		
5	Low	7.9(8th)		
	Low	5.9	---------	------
	10-94	3-93		
6	Low ->Moderate	12+		
	Moderate	8.5	---------	------
	10-93 3-94	3-94		
7		9.3		
	------	5.6	---------	------
		3/94		
8	No Information Available		---------	------
9	Moderate	11.6		
	Low	9.6	---------	------
	3-94	3-94		
10	Moderate	9.4		
	Moderate	12+	---------	------
	3-94	10-92		
11	Low	9.6		
	Low	9.3	---------	------
	10-94	3-94		
12	Moderate	9.4		
	Moderate	12+	---------	------
	10-93	3-94		

				(TABE
13	Moderate			12+
	Satisfactory	------	------	12+
	10-93			8-92)
14	Moderate	8.9	10	
	Moderate	6.1	8	------
	10-94	3-94	8-93	
15				13.5
	------	------	------	13.5
				4-95
16	Low	11.2		
	Satisfactory	12.9	---------	------
	10-94	3-95		
17	Satisfactory (7th)			
	Satisfactory (7th)	---------	---------	------
	10-92			
18	Low	9.6		
	Moderate	11.0	---------	------
	3-95	3-94		
19	Satisfactory (7th)			
	Satisfactory (7th)	------	---------	------
	10-92			
20	Moderate (7th)		7	
	Satisfactory (7th)	------	5	------
	10-90		8-94	
21	No Information Available		---------	------
22	Moderate	7.9		
	Low -> Moderate	8.3	---------	------
	10-94 10-95	4-94		
23	Moderate (7th)			
	Moderate (7th)	------	---------	------
	10-92			
24	Low	6		
	Low	8	---------	------
	10-95	4-94		
25	Low			
	NA	------	---------	------
	10-94			

26	Low Low 3-96	------	---------	------
27	Satisfactory Moderate 10-95	------	---------	------

All grade levels are listed with reading on top and math in the middle. The date that the test was administered is on the bottom.

MEAP math test results are described on the range Low-Moderate-Satisfactory

MEAP = Michigan Educational Assessment Program
CAT = California Achievement Test
Brigance = Brigance Inventory of Basic Skills
Iowa= Iowa Survey Of Basic Skills
TABE = Adult Basic Education