Exploration of Design Microbiology Experiments: Taking the Separation and Purification of Microorganisms in Soil as an Example

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Abstract: Microbiology experiment is a professional basic course for biotechnology majors. This course focuses on basic experiments. In order to cultivate students' innovative ability and scientific research literacy, individual basic experiments are changed to design experiments. Taking the separation and purification of microorganisms in soil as an example, this experiment uses the student experimental group as the unit to design the experimental plan independently, carry out the experiment in a combination of classroom and open laboratory, the assessment and evaluation of the results in a combination of diversification. With a complete trial run of 2 teaching cycles, the teaching method tends to be mature, with certain innovation, feasibility and generalizability. It is helpful for cultivating students' innovative thinking ability, independent analysis and problem-solving ability and hands-on operation ability, and Improving students' comprehensive experimental quality has a significant effect.

Keywords: Designing experiment; Soil; Microbiology; Isolation; Purification

1. Introduction

Microbiology is an experimentally based natural science that is required of students majoring in biology, agriculture, and soil science in higher education. It has a high standing in the curriculum and helps students advance their careers. Microbiological experiments are crucial to the entire life sciences education process because they help students grasp academic courses better and develop their practical skills [1-2]. It is very applicable and experimentally sound. In addition to learning fundamental concepts and abilities, students must to be able to use creative problem-solving techniques on their own. In order to foster students' inventive thinking, scientific research thinking, and thorough application of information, fewer validation studies and more design experiments are conducted. Through the experimental course, students can enhance their comprehension and application of theoretical knowledge as well as strengthen their analytical, problem-solving, and practical thinking skills. In addition, students will learn to seek truth from facts and cultivate a spirit of scientific inquiry [3].

The primary environment for microbial development is soil, and soil microorganisms, which are abundant and have a very complex makeup, are a crucial part of ecosystems. They are a general word for the majority of the bacteria, fungi, actinomycetes, and algae that reside in soil [4,5].

Seventy to ninety percent of all soil microorganisms are bacteria. They make up the greatest living surface and most active living element in the soil because of their tiny size, high abundance, and especially large surface area in touch with the soil. They exchange materials with their surroundings on a continuous basis. Furthermore, the most active microbial community in the rhizosphere soil is found in the rhizosphere of plants, which is a soil microzone affected by plant roots. This community mostly consists of a variety of microorganisms, including bacteria, actinomycetes, fungus, and others. "Separation and purification of microorganisms in soil" is the focus of this experimental project, which uses a fully open learning environment. Various types of soil are utilized as test materials, and students independently design experimental plans and develop plausible experimental routes using lab equipment and a

thorough review of the literature to obtain techniques for the separation and purification of microorganisms in soil.

2. Experimental Procedure

The preceding experiment incorporated microbiology, took place during the last semester of a junior biology major.

Make five groups of four to five kids each, one week before to the experiment. Following a discussion, the experimental plan is submitted to the teacher for review. The teacher will inform the students of the questions and experiment's content, as well as the materials available in the library and on the Internet. The group designed the experimental program and each member is responsible for their own work, which includes preparing the soil prior to collection, the type of soil collected, the depth of the collection, methods for treating the soil, and so forth. After the teacher and students have a debate, the final draft of the experimental plan is chosen.

Start the experiment. Every group member will begin the experiment based on their own strategy. The trial ran for two to three weeks. In order to assure the smooth operation of the experiment, the safety of the laboratory, and the resolution of any issues that students could experience at any moment, the experiment was conducted in an open laboratory due to its nature and the discontinuity of its scheduled time. Students' demands for equipment for design experiments can be satisfied by the shaking table, sterile operation table, biological incubator, inverted fluorescence microscope, and other hardware facilities. Team members work well together, create good experiments at every stage of the record, and communicate with professors via WeChat or QQ. The group can talk at any moment.

Depending on the experimental conditions, each group's members must write a brief paper or create a PowerPoint presentation at the end of the experiment. Regardless of format, the experimental report must include the five parts of content, including the purpose, principles, content, results, discussion, and conclusion. 20% went toward innovation, 40% went toward problem-solving skills, 10% went toward cooperation and division of labor, 20% went toward the analysis being in place, 10% went toward writing a paper, PPT, or experiment report, and 10% went toward the experiment's true and reliable outcome.

3. Concrete Experiment

3.1 Create a Plan:

Following extensive debate between the teacher and the students, the final draft and explicit directions for carrying out the experiment are created. Students combine their theoretical knowledge in answer to the teacher's questions, go to the library and reading room, and study experimental manuals on microbiology, environmental microbiology, food microbiology, cell biology, and current research.

The experimental scenario is shown in Figure 1.

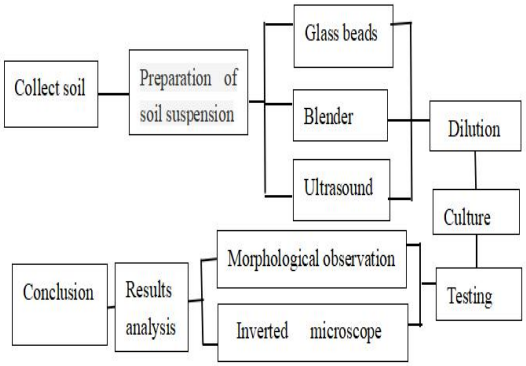

Figure 1. Studies the Flow

Each experimental group gathered various soil types (clayey loam, sandy loam), made soil suspensions using various techniques, diluted and cultured the soil, inspected it using an inverted microscope for morphological observation and Gram staining, took pictures, examined the findings, and made a conclusion.

Prior to the experiment, students should have a firm grasp of the fundamental ideas and procedures involved in separating and purifying microorganisms from soil. They should also have furthered their understanding of the inverted plate method and the fundamental operational procedures of linear plate separation. Microbes coexist in diversity in nature.

A microbe needs to be separated from the mixture and cultured in order to be studied or used. The culture conditions must be tailored to the specific needs of the microbe, taking into account its ph, oxygen, and nutrition requirements. The other bacteria must be removed, and the pure strain [6,7] can be

obtained by dilution, coating, line-drawing, and other methods of separation, purification, and single colony formation.

Experimental equipment and material equipment: horizontal shaking table -LRB-model: ZQTY-70ES/ZHTY-70ES, manufactur Shanghaig Zhichui Instrumentm Co. coLtd. ltd. Asepticp Operationt Tablea-LRB-le (model: SW-CJ-2D, manufa Suzhou: Purificationf Equipmentq Co. menLtd, ltd.), ultrasonic (model: ZL6-180D ultrasonic cleaning machine), biological incubator, electronic balance, etc. Material: soil sample, culture dish, glass bead, triangle bottle, test tube, etc.

3.2 Experimental Methods

(1) The medium for separating bacteria and Actinomycetes is prepared as follows: 3 grams of beef extract, 10 grams of peptone, 5 grams of sodium chloride, 1000 milliliters of distilled water, 15 grams of agar powder, and a pH modulation of 7.0 to 7.2 are all added to a flask and sterilized at 121 degrees Celsius for 20 minutes. Following sterilization, the medium is cooled to 50 to 60 degrees Celsius in a sterile operating table near an alcohol lamp. Once the medium is set, the culture dish is turned upside down for two to three days. Afterwards, carry out a follow-up operation, making sure the culture medium is free of pollution and discarding it if necessary.

Gaoshi is the medium used to isolate actinomycete 20 grams of soluble starch, 1 gram of potassium nitrate, 0.5 grams of potassium dihydrogen phosphate monohydrate, 0.5 grams of magnesium sulfate heptahydrate, 0.5 grams of sodium chloride, 0.01 grams of anhydrous iron (2+) sulfate heptahydrate, 1000 milliliters of distilled water, 15 grams of agar powder, and a pH modulation of 7.0–7.2 make up my medium. The culture media is subpackaged and sterilized in the same way as the bacterial culture medium.

Making soil diluent for each experimental group. Each group was selected from a separate soil sample based on their own experimental program. Following the removal of debris from the soil's surface, each sampling point had its soil sections dug out. Two soil layers—0–20 cm and 20–40 cm—were then sampled with knives. The soil samples from three sampling points and one soil layer from the same plot were then evenly mixed and placed in an aseptic bag. The soil samples were kept for a maximum of 24 hours before being placed in an ice pack into a foam box and sent back to the lab for prompt processing.

(2) After the soil samples were taken back to the laboratory, plant roots and rocks were removed, and the soil samples were randomly divided into 3 parts and placed in a 4 ° C refrigerator.

1-3 groups of sandy loam, 4-6 groups of clay loam, each group of the same soil sample preparation of soil suspension method is not the same, the first group called 10 g of soil samples, put into sterile bottle, add glass beads and 90 ml sterile water, shake on a shaking table for 20 minutes, let stand, the soil completely sink, bacteria completely dispersed, with a sterile gun to absorb 1 ml of soil solution, into a test tube containing 9 ml of sterile water, mix, then absorb 1 ml soil mixture to dilute the same method, mixing. A soil solution of 10-1-10-6 dilution was prepared. The second group mixed the soil solution with a mixer, the third group mixed the soil solution with ultrasonic wave, other operations as the first group. Groups 4-6 are operated in the same way as groups 1-3.

(3) Coating. 0.1-0.2 ml soil suspension with different dilution was added to the solid separating medium plate of bacteria and actinomycetes, spread evenly, and placed at room temperature for 5-10 min. The plate of bacteria isolation medium was inverted in 37 ° C incubator for 1-2 days, and the plate of actinomycete isolation medium was inverted in 28 ° C incubator for 3-5 days. The same dilution 3 times in parallel. After isolation and purification, the single colonies were transferred to the inclined surface of the tube and stored at 4 °C.

(4) Description and identification of the strain When the strain was cultured for 24 h, the growth rate of the colony was significantly higher than that of the control group, the medium growth rate of the colonies was observed, while the slower growth rate was observed when the strains were cultured for 48 H-72 H, the colony size, shape, color and edge shape were observed.

3.3 Results

The average colony number (bacteria and actinomycete colony number) of the same dilution in sandy loam soil of 0–20 cm and 20–40 cm was clearly affected by the depth of soil

layer; the colony number of 0–20 cm soil layer was clearly higher than that of 20–40 cm soil layer, and the 20 cm soil layer was simply the crop's rhizosphere. In the 20–40 cm soil layer, the colony number is typically 2-3 when the soil solution is diluted to a 10–5–10–6 ratio.

The treatment method of soil suspension affects the average colony number (bacteria and actinomycete colony number) of the same dilution in clay soil of 0–20 cm layer and 20–40 cm layer. Glass beads are not as effective as ultrasonic waves in this regard. The effect of soil depth on microorganisms was similar to that of sandy loam.

3.4 Experimental Results

The variety of methods used to collect the soil sample, the depth of the soil layer, the method used to treat the soil sample, the separation and purification of the bacteria and Actinomycetes, the observation of colony morphology, counting, diameter measurement, physiological characteristics, Gram staining, and microscopic observation all contribute to the rich content of the experiment that piques students' interest and broadens their knowledge. In the past, there were very few experimental contents in microbiological experiments due to time constraints on the instructional material.

Additionally, the experimental teachers prepared the soil samples as well, and there weren't many hands-on activities for the pupils. While there were no strict guidelines for staining and microscopic examination, some students chose to wash the germs after witnessing them develop. There was not enough time in class to finish everything. Now things were not the same. Once the lab opened, students may apply to work there during their free time, and they were the ones who developed the experiment from the start. Naturally, they needed to be given an explanation of the experiment's findings.

As a result, according to the experiment report, the students did not want to miss any steps in the process, and the teacher's grading guidelines had changed. Instead, they wanted to see the experimental procedure and the outcomes, which also encouraged the students to experiment with a different mindset. Rich content, but also make use of the students' theoretical expertise to enhance their comprehension. The integration of theoretical knowledge with practical application established a foundation for scientific research and application-focused people training [8].

Students are curious about how the soil season affects them, which was piqued by the fact that the same soil solution, because of the difference in the media, allowed the isolation of different microorganisms and whether different types of microorganisms were different in different crop types. Some interest groups conduct studies outside of the classroom by tracking the seasons and sorts of crops. Some even apply to research projects at universities where they want to perform in-depth investigation.

What happens to the soil's microorganisms when industrialization pollutes it? The study group's students also focused on gathering and analyzing soil samples from nearby farms in order to conduct experiments. The students feel that the single description of morphology is insufficient; some of them have approached the subject from the perspective of molecular biology, understanding microbes at the molecular level through the extraction of DNA from bacteria. This has provided a solid foundation for their graduation theses and subsequent courses.

The grouping experiment fostered the kids' sense of cooperation.

Two grouping strategies were used: grouping by student number order and grouping by free combination. Some students spontaneously established groups since their interests were comparable, they are well-known to one another, and they can cooperate and divide labor well. A subset of students organize into teams based on their numerical order.

They are divided into a group with mutual tolerance, unity and cooperation, and division of labor; they are no worse than the free combination, collective honor, and overall awareness, and they are all performing better. At the same time, it has formed a good inheritance of excellent students' initiative to help backward students, and it has practiced the spirit of team cooperation and the cultivation of overall situation awareness of college students in the new era. Their interests and personalities differ more than those of free combinations.

4. Conclusion

Teaching microbiology through experiments is not only a means of demonstrating and

expanding theoretical knowledge, but also a window and a platform for developing students' capacity for independent thought, creativity, and innovation, as well as their capacity for practical application, teamwork, and scientific reasoning [9,10].

An essential foundational subject, microbiology experiments are a vital component of all life-related courses in nearby application-focused colleges. Students' attention to the experimental lesson is increased, their learning potential is stimulated, and their experimental skills are exercised through the well-designed microbiology experiment. Additionally, the students are trained to think independently, analyze, synthesize quality, and think like scientists conducting research.

They are experts in writing scientific research papers and conducting literature reviews. Through the planned experiments, the students' methodical comprehension and mastery of microbiological knowledge were strengthened. This is important for the application of biology research projects, participation in various life science competitions, Internet+, and provincial and national innovation projects. The future of their graduation thesis, postgraduate entrance examination, or re-examination to the scientific research unit employment has a significant aid because of their scientific research talent and thinking training.

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