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Fecal Escherichia Coli Multi-Tube Fermentation, Fecal Escherichia Coli Filter Paper and Vicia Bean Root Tip Micronucleus Method were Used to Detect Water Quality

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Abstract: The rapid development of social economy, the continuous improvement of the level of industrialization, in promoting the progress of human society at the same time, but also brought some environmental problems, especially water pollution problems, not only endanger people's life and health, but also affect People's Daily life. "Clear water and green mountains are gold and silver mountains", green ecological development is also an important guideline of the country, environmental protection is urgent. And strengthening water quality testing is of great significance to ensure the environment. Water is the source of life, whether in daily life or industrial and agricultural production, water is an indispensable resource. In the process of protecting water resources and controlling water pollution, water quality testing, as an effective way, can meet the requirements of social production and life on the use of water resources. To this end, this paper simply discusses the fecal Escherichia coli multi-tube fermentation detection, fecal Escherichia coli filter paper detection, bean micronucleus detection of water quality detection methods. [1-3]

1. Fecal Escherichia Coli Multi-Tube Detection Method

1.1. Experimental Principle

Fecal coliforms are part of the total coliforms in the microbiology program and are used primarily to indicate the extent of fecal contamination of water quality. Fecal coliform is a kind of anaerobic and facultative anaerobic Gram-negative bacillus, which can ferment lactose to produce acid and gas. Cultured at 44.5 ℃ for 24 to 48 h, it can ferment lactose to produce acid and gas. The level of fecal coliform in water body is closely related to the surrounding economic development, the influence of enterprises, population and other factors. [16]Exceeding the standard of water body will affect the ecological environment of the whole basin, which is extremely adverse

to human health. By monitoring faecal coliform, we can know the status of water polluted by domestic sewage. At present, WHO, ISO and most countries in the world take fecal coliforms as indicators of fecal pollution in water quality [12]. Fecal coliform is applicable to the monitoring of river, lake and other surface water, enterprise sewage and hospital wastewater, and is an essential and important index for comprehensive evaluation of urban sewage pollution, especially domestic sewage pollution[6-10].

1.2. Experimental Procedure

(1) Water sample inoculation: In strict accordance with the national standard requirements, the water sample is fully mixed and the inoculation amount is determined according to the sample concentration. Each sample is inoculated with 3 different water samples, and the same water sample is inoculated with 5 tubes. (2) Initial fermentation experiment: the water samples were inoculated into fermentation tubes containing lactose protein culture medium and cultured at 37 °C ±0.5 °C for 24 h. The fermentation tubes producing acid and gas showed positive results. [11]If the gas production in the tube is not obvious, tap the tube and small bubbles rise to indicate positive. (3) Re-fermentation experiment: slightly shake the fermentation tube with positive results of the initial fermentation experiment, and transfer the culture to the EC medium with 3mm inoculation ring. The culture was incubated at 44.5 ℃± 0.5 ℃ for 24 h. The results were judged immediately after culture. [1]

1.3. Determination of Experimental Results

1.3.1. Schematic Diagram of Gram Staining

Gram-positive bacteria are shown in Figure 1; Gram-negative bacteria are shown in Figure 2

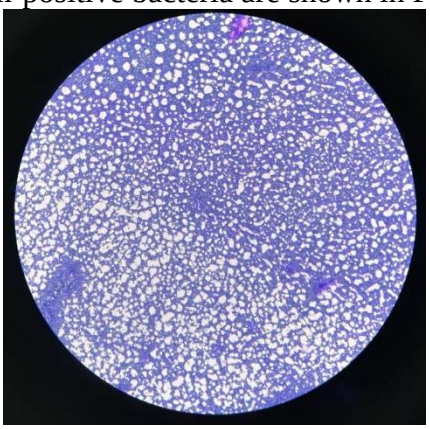

Figure 1: Gram-positive bacteria

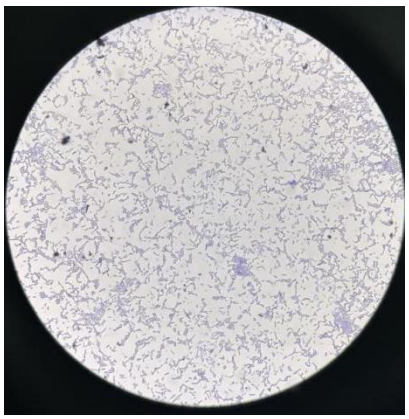

Figure 2: Gram-negative bacteria

1.3.2. Determination of Experimental Results

The sample was added to the test tube containing lactose peptone medium, and the initial fermentation and enrichment culture was carried out at 37℃. Coliform group grew and multiplied in the medium to decompose lactose and produce acid and gas. The acid produced made bromocresol purple indicator change from purple to yellow, and the gas produced went into the inverted tube to indicate gas production. [11-13] 44.5 ℃ for further fermentation culture, bile salt 3 in the medium can inhibit the growth of gram-positive bacteria, and finally the bacteria producing

gas was determined to be fecal coliform. [17-19] The concentration of fecal coliform was obtained by checking the MPN table.

1.4. Advantages and Disadvantages of the Experiment

The determination of faecal coliform requires initial fermentation test (enriched culture at 37℃ for 24 h, coliform multiplies in the medium and produces acid and gas) and re-fermentation test (cultured at 44.5℃ for 24 h, bile salt III in the medium can inhibit the growth of gram-positive bacteria. The positive results of faecal coliform were illustrated by gas production in the refermentation tube). [15]The total coliform needs lactose fermentation test (24±2 h in an incubator at 36±1 ℃), plate isolation culture (the fermentation tube producing acid and gas is transferred to a red methylene blue AGAR plate and cultured in an incubator at 36±1 ℃ for 18 ~ 24 h) and verification test (The Gram-negative bacillus pastris detected through staining microscopy was inoculated into lactose peptone medium and then cultured for 24±2 h in an incubator at 36±1 ℃) in three steps, requiring 72 h to obtain the results. Moreover, the verification test was complicated and there were many interfering factors.

2. Fecal Escherichia Coli Filter Paper Method

2.1. Experimental Principle

Each sample was inoculated with 3 different 10-fold decreasing inoculation amounts, and 5 pieces of paper were inoculated with each inoculation amount, a total of 15 pieces of paper were inoculated. The inoculation amount was determined according to the contamination degree of the water sample. As far as possible, the 5 pieces of paper with the largest inoculation amount were positive and the 5 pieces of paper with the smallest inoculation amount were negative, so as to avoid the occurrence of 15 pieces of paper with all 3 different inoculation amounts being positive or negative. The inoculated samples were cultured at 44.5 ℃± 0.5 ℃ for 24 h and observed to interpret the results. [14]

2.2. Judgment of Experimental Results

2.2.1. Experimental Culture Results are Shown in Figure 3

Figure 3: The results of fecal Escherichia coli filter paper culture

2.2.2. Measurement of Experimental Results

The samples were filtered through a filter membrane with a pore size of 0.45 μm, and the bacteria were trapped on the filter membrane. Then the filter membrane was placed on the MFC selective medium and cultured at a specific temperature (44.5 ℃) for 24 h. Bile salt No. 3 could inhibit the growth of gram-positive bacteria. Fecal coliform bacteria can grow and ferment lactosugars to produce acid to change the color of the indicator. Acid production can be determined by color. The concentration of fecal coliform bacteria in the sample can be measured by counting the colonies that are blue or blue-green. [14] 1.2 Water samples are collected about 10 ~ 15cm from the water surface. The samples are collected in containers, and a certain amount of water samples are inoculated on specific sterile filter paper in aseptic operation, and cultured at a specified temperature (37℃ or 44.5℃) for 24 h. The presence of total coliform or faecal coliform was determined by the color change of the indicator (bromoviolol purple indicator changed from purple to yellow, and red spots were shown in the yellow background after acid production), and then the concentration of total coliform or faecal coliform was obtained by checking the MPN table.

2.3. Advantages and Disadvantages of the Experiment

Among the detection methods of fecal coliform, the multi-tube fermentation method is the traditional detection method, which has many interference, poor specificity, complicated operation and time consuming, and requires confirmation experiment, so it is not suitable for the analysis of a large number of samples. However, the traditional method has the advantages of simple principle, low cost and easy to popularize, so it is still the main detection method in routine monitoring. Compared with the multi-tube fermentation method, the disk method only needs to be cultured at 44.5 ℃ for 18 ~ 24 h to get the results. The disk method is fast, time-saving, simple and convenient, suitable for mass monitoring and emergency monitoring, but the test results are not as accurate as the multi-tube fermentation method. [20]

3. Micronucleus Detection of Broad Bean

3.1. Experimental Principles

Micronucleus (MCN) is an abnormal structure in eukaryotic cells, often produced by cells through the action of radiation or chemical drugs. In the intercellular phase, micronucleus are round or oval, free outside the main nucleus, and the size of MCN should be 1/3 lower than the main nucleus. It is generally believed that in the process of chromosome replication, due to the influence of pollution factors, the broken chromosome fragments do not have centromere. At the end of cell division, the fragments are lost into the cytoplasm and become micronucleus. [14] Vicia faba root tip micronucleus technique is a plant micronucleus detection method with chromosome breakage and spindle damage as the test endpoint. It is accurate, rapid, easy to operate, has obvious dose-effect relationship, and is suitable for the detection of large quantities of samples. Besides, the number of micronucleus produced is proportional to the strength of the dose of mutagenesis factor. Therefore, micronucleus test, as an effective biological short-term test method, is applied to the monitoring of water pollution, which is listed as a routine index in many countries, and included in one of the standardized methods of water environment monitoring.

3.2. Experimental Procedure

① Soak seed and promote germination. Select full particles and uniform size of broad bean seeds, washed with distilled water for 2 ~ 3 times, respectively, placed in the plate equipped with a variety of water samples, placed in (25 ± 1) ℃ incubator culture for 1 ~ 2 days. The negative control group was cultured with tap water all the time; Positive control group was cultured with tap water for 72 h and then with 0. 01% HgCl2 was soaked for 1 h, washed and then cultured in tap water. Broad beans in experimental group were soaked in tap water for 24 h before being cultured in groups with each water sample. ② Root tip fixation. Long young roots 1. 5 ~ 2. After 0 cm, the root tip was cut off 1.0 cm with a blade and fixed for 24 h with the newly prepared Carnot's fixating solution (methanol: glacial acetic acid = 3： 1), then washed with 95% alcohol and transferred to 70% alcohol for storage. ③ The material was dissociated. The root tips were dissociated with 1 mol /L HCl for 15 min, and were further dissociated in a water bath at 60 ℃ for 8 ~ 10 min. ④ Film. Stain with basic fuchsin for 20 min, rinse with tap water, and then use the tablet pressing method. ⑤ Routine microscopic examination: 3 root tips were randomly examined for each water sample to be tested and the control group. At least 1 000 cells of growth points were observed for each root tip under a high power microscope, and the number of cells with micronucleus was recorded, and the micronucleus rate of the test group and the control group were respectively compared. [16]

3.3. Determination of Experimental Results

3.3.1. Judgment of Micronucleus

The micronucleus has the same coloring. When turning the fine-tuning, it is on the same horizontal plane as the main nucleus; Round or oval, well delimit, contained in the cytoplasm, diameter less than 1/3 of the diameter of the main nucleus, and not connected with the main nucleus; The micronucleus is intact and does not overlap, except in dead or degraded cells. [5] The micronucleus is shown in Figure 4.

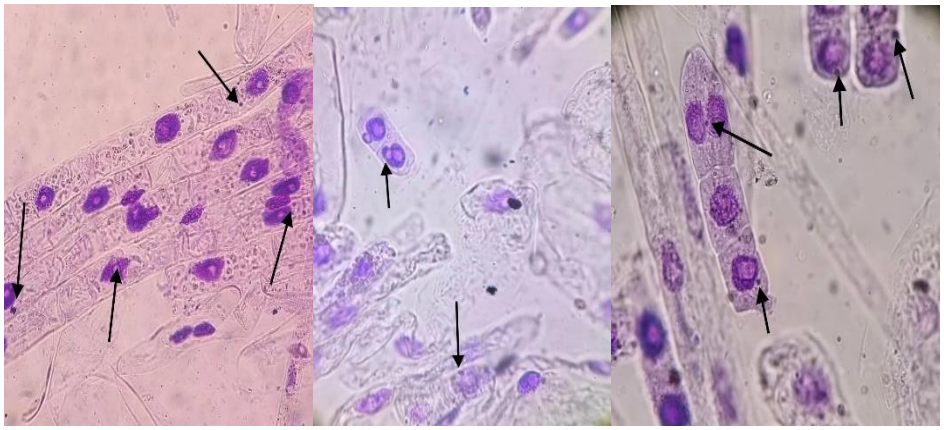

Figure 4: The results of micronucleus experimen

3.3.2. Calculation of micronucleus millage rate (MN) ‰ of experimental test samples (including control group) according to the following formula. [7]

$$MN\ ‰ = MC \times 1000 / TC$$

The symbol description is shown in Table 1

Table 1: The formula conformity

MCN ‰	Micronucleus rate
MC	Look at the number of cells with micronucleus in the cell
TC	Total number of cells observed
PI	Pollution index

Pollution Index (PI) = sample test mean MN‰ / control mean MN‰

According to the pollution index classification standard stipulated in "National Biotechnology Inspection Standard - Vicia Bean root Tip Micronucleus Technology", pollution number (PI) value is used to classify the pollution degree, and pollution index value is used to evaluate water quality standard pollution index value to judge water quality according to pollution index. This method can avoid MN‰ fluctuation caused by test conditions and other factors, so it is suitable for application. [4]The judgment of pollution index is shown in Table 2

Table 2: The judgment of pollution index

MN ‰ value	Pollution degree
0 < MN ‰ < 1.5	Basically pollution-free
1.5 <MN ‰< 2.0	Light pollution
2.0 <MN ‰< 3.5	Moderate pollution
3.5 <MN ‰	Severe pollution

3.4. Advantages and Disadvantages of the Experiment

Domestic and foreign scholars have conducted a large number of experiments and studies on the root tip detection technology of broad bean, and a mature application system has been formed. As a standard plant for international genotoxicity studies, the pollution index is taken as the standard for the level of environmental pollution. The range of environmental pollution increases with the increase of micronucleus millage rate and pollution index.

4. Conclusion

Environmental protection is the cause of contemporary, benefit in the future, and water quality testing plays a very important role in it. Only on the basis of following the national environmental protection policy, rational use of water resources, in order to give full play to the maximum promoting effect of water resources on social, economic and human development. Water quality testing has effectively promoted the supervision of environmental protection. By providing accurate water quality testing data and technical support, it provides effective proof materials for environmental protection law enforcement. In the process of water quality testing, we should start layer upon layer from the sampling link, improve the scientific and accuracy of water quality testing, and never let any behavior damaging the environment go unpunished. The combination of powerful environmental protection administration law enforcement means, to achieve the ultimate goal of environmental protection.

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