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Study on infection of blueberry explants with different antibiotics

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Abstract. The effects of streptomycin sulfate, carbendazim, penicillin and nystatin on the inhibition of fungi and bacteria in blueberry explants were studied. The results showed that the addition of streptomycin sulfate or penicillin with different concentrations in the medium had a certain inhibitory and killing effect on bacteria in blueberry explant contamination, while the addition of carbendazim or nystatin had a certain inhibitory and killing effect on fungi in blueberry explant contamination. When the concentration of streptomycin sulfate in the medium reached 60units / mL, the highest survival rate was 44.0%, and the bacterial infection rate was the lowest. When the concentration of carbendazim in the medium was 0.4 mg/mL, the survival rate reached 40.5%, and the fungal infection rate decreased greatly. When the penicillin concentration in the medium reached 40mg/mL, the lowest infection rate of bacteria was 4.0%, and the highest survival rate was 31.5%. When the concentration of nystatin in the medium reached 40mg/L, the highest survival rate was 34.5%. Therefore, streptomycin sulfate had better inhibitory effect on bacteria, while carbendazim had better inhibitory effect on fungi, which provided reference for future research.

1 Introduction

Plant tissue culture technology has very strong practicability in contemporary society. It is used in different fields and has very strong application value in scientific experiments and production[1]. However, the widespread existence of microorganisms has become a major obstacle to the development of tissue culture technology. In the process of explant culture, it is easy to be contaminated by bacteria or fungi in the environment, thus affecting the survival rate of tissue culture, which requires us to find appropriate antibacterial methods in the experimental process.

Antibiotics have significant inhibitory effects on bacteria and fungi in plant tissue culture. Antibiotics are bioactive substances produced by some organisms, which are resistant to pathogens or have other effects, and can inhibit the development and metabolism of other microorganisms[2]. Antibiotics mainly inhibit bacterial cell wall synthesis, protein synthesis, nucleic acid synthesis and affect cell membrane function to achieve antibacterial effect. With the continuous progress of society, antibiotics are more widely used in the prevention and treatment of explants pollution.

In order to study the effects of different concentrations of antibiotics on the infection of explants, fresh blueberry explants were used as culture materials, and different concentrations of streptomycin sulfate, carbendazim, penicillin and nystatin were added in the medium. The effects of different concentrations of antibiotics on the infection of blueberry explants were studied by experimental observation. The data were recorded to find the optimal concentration gradient of different antibiotics.

2 Materials and methods

2.1 Material

The vigorous growth of blueberry shoots were used.

2.2 Reagents

Streptomycin sulfate, carbendazim, penicillin, nystatin, and WPM medium were used.

2.3 Methods

2.3.1 Selection and treatment of explants

The selection of explants mainly depends on leaves, stems, tender buds, green branches, among which stems and leaves are more common. Blueberry explants should be collected in sunny days without water droplets in leaves[3]. Cut blueberry explants into stem segments with scissors. Wash them in the pool for 30min, and then wash them with detergent for 5min without stirring. Wash them with tap water for 60min to remove detergent residue. Then soak in 70% alcohol for 30s. Rinse with sterile water for 3 times. Use 0.1% mercury chloride disinfectant for 9min[4]. And finally clean and dry for later use.

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2.3.2 Preparation and sterilization of medium

The WPM medium was used as the basic medium, and the concentrations of streptomycin sulfate were 20, 40 and 60 units/mL respectively. The concentrations of carbendazim were 0.2, 0.4 and 0.6mg/mL respectively. The concentrations of penicillin were 20, 30 and 40mg/L respectively. The concentrations of nystatin were 20, 40 and 60 mg/L respectively. The pH was adjusted, and the medium without antibiotics was used as the control.

In addition, agar needs to be heated and dissolved at high temperature in an electromagnetic furnace, heated and stirred, and finally sucrose is added to the medium. In autoclave, the medium was sterilized at 121 °C and 20-30min. In addition, the tweezers and scissors used in the experiment also need to be sterilized, and they should be wrapped in newspapers and ropes. Avoid breakage and prevent infection.

2.3.3 Ultraviolet sterilization

The medium was removed from the autoclave, cooled to room temperature, and then the medium, forceps, scissors and blueberry explants stem segments were placed into the ultra-clean working table for UV sterilization for 20min.

2.3.4 Inoculation and cultivation

Before the experiment, the hands were disinfected with 75% alcohol, and then the alcohol lamp was lit. The tweezers and scissors were burned in the external flame. After cooling, the blueberry stems were cut into 2-3cm and put into the medium. During the inoculation, the tweezers should not touch the surface of the medium. Before the medium was opened, the edge should be burned by flame, and after the inoculation was completed, the flame should be burned again to prevent infection. The inoculated medium was labeled and cultured in the suitable environment of 16-18°C[5].

2.3.5 Results Observation and data processing

Explants need to be observed every day for 7 days. The infection rate and death rate of different concentration gradients should be recorded in detail, and finally counted and analyzed.

3 Results and analysis

3.1 Effects of different concentrations of streptomycin sulfate on infection of blueberry explants

The experimental data showed that the addition of streptomycin sulfate in the medium could significantly reduce the bacterial infection of blueberry explants. With the increase of streptomycin sulfate concentration, the bacterial infection rate decreased to 13%, and the range was large. However, the fungal infection rate began to decrease, but when the concentration was 40units/mL, the fungal infection rate increased, indicating that streptomycin sulfate had a significant inhibitory effect on bacteria, and had no significant effect on fungi.

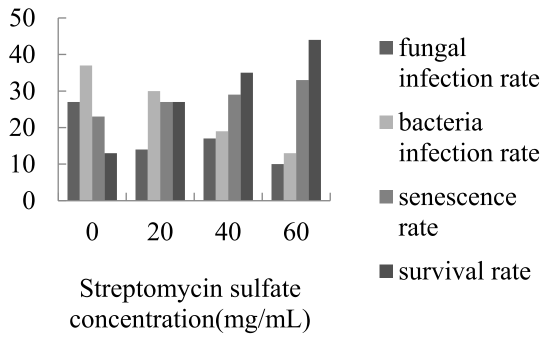

Fig.1 Antibacterial effect of streptomycin sulfate on blueberry explants

In this experiment, it can be seen from the addition range of streptomycin sulfate that the addition of streptomycin sulfate can increase the survival rate by at least 14%. Streptomycin sulfate has certain inhibitory effect on bacteria, but it also has certain damage to blueberry explants. The dead rate increased with the increase of streptomycin sulfate concentration, and the dead rate of explants without streptomycin sulfate was the lowest. So the optimal concentration of streptomycin sulfate in the medium was 60units/mL, and the highest survival rate was 44%.

3.2 Effects of different concentrations of carbendazim on infection of blueberry explants

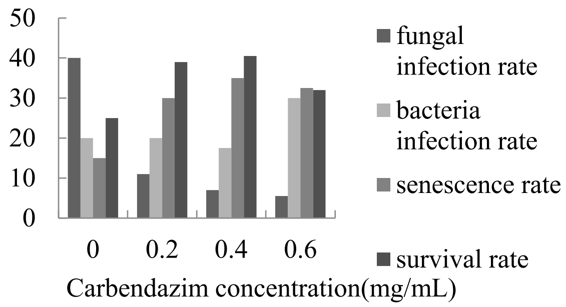

Fig.2 Antibacterial effect of carbendazim on blueberry explants

According to fig.2, the fungal infection rate decreased significantly with the increase of carbendazim concentration, from the initial 40.0% to 5.5%, and the variation was large, indicating that the effect of carbendazim on inhibiting fungi was particularly significant. When the same concentration of carbendazim was added, the bacterial infection rate did not change at the beginning. Then, with the increase of concentration, the bacterial infection rate even increased from 17.5% to 30.0%, indicating that carbendazim could effectively act on fungi and had no effect on bacteria. The data showed that the mortality rate increased with the increase of carbendazim concentration, which was positively correlated, and increased by at least 15.0%. When carbendazim was not added, the death rate was the lowest, which also reflected that antibiotics acted on pathogens, and at the same time, they also had certain damage to blueberry explants themselves. Therefore, we should not blindly pursue to reduce the infection rate, but also pay attention to the survival rate. In this experiment, when the concentration of carbendazim was 0.4mg/mL, the highest survival rate was 40.5%, and the fungal infection rate was significantly reduced.

3.3 Effects of different concentrations of penicillin on infection of blueberry explants

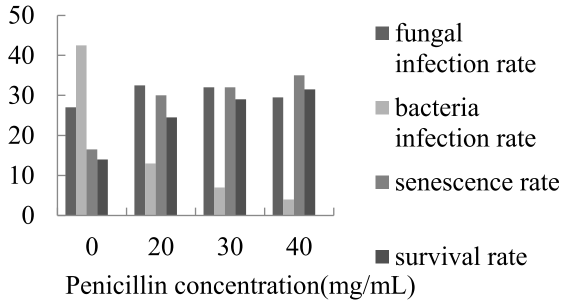

Fig.3 Antibacterial effect of penicillin on blueberry explants

According to fig.3, after adding penicillin, the change of bacterial infection rate was particularly obvious, the initial without adding penicillin showed 42.5%. With the increase of penicillin concentration gradient, the bacterial infection rate eventually decreased to 4.0%, and the change was large. The bacterial infection rate was negatively correlated with the concentration of penicillin, but the fungal infection rate did not change with the increase of concentration. Penicillin mainly acts on bacteria and has no obvious inhibitory effect on fungi. When the concentration of penicillin reaches 40mg/L, the lowest infection rate of bacteria is 4.0% and the highest survival rate is 31.5%. At the same time, according to the data in the table, the mortality rate was positively correlated with the addition concentration of penicillin. When penicillin was not added to the medium, the death rate was the lowest, but the survival rate was also the lowest, indicating that bacterial pollution was serious. With the increase of penicillin concentration, the death rate gradually increased. However, due to the effective inhibition of bacteria by penicillin, the survival rate increased. Therefore, when the penicillin concentration reached 40mg/L in this experiment, the infection rate of bacteria was the lowest, and the survival rate was the highest of 31.5%.

3.4 Effects of different concentrations of nystatin on infection of blueberry explants

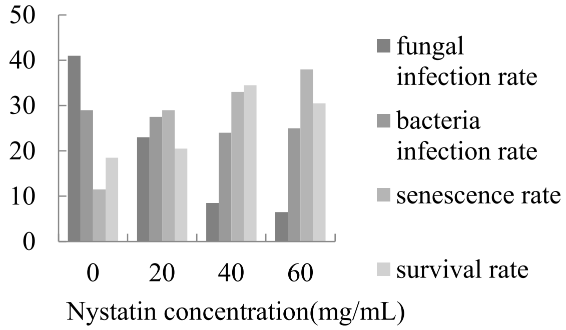

Fig.4 Antibacterial effect of nystatin on blueberry explants

The results showed that the fungal infection rate gradually decreased with the increase of nystatin concentration. When the concentration reached 60mg/L, the fungal infection rate of explants was the lowest of 6.5%. This indicated that nystatin could effectively act on fungi, but had no obvious effect on bacteria. According to the data, when no nystatin was added, the lowest dead rate was 11.5%, and the dead rate was proportional to the concentration of nystatin. When the concentration reached 60 mg/L, the dead rate was the highest. The optimal concentration of nystatin added to the medium in this experiment was 40 mg/L, and the highest survival rate was 34.5%.

4 Discussion and conclusion

Adding streptomycin sulfate or penicillin in the medium had a significant effect on the bacteria in the pollution of blueberry explants, while carbendazim and nystatin had a certain killing effect on the fungi in the pollution of explants. When the concentration of streptomycin sulfate in the medium reached 60units/mL, the highest survival rate was 44.0%, and the bacterial infection rate was the lowest. When the concentration of carbendazim in the medium was 0.4mg/mL, the survival rate reached 40.5%, and the fungal infection rate decreased greatly. When the penicillin concentration in the medium reached 40mg/L, the lowest infection rate of bacteria was 4.0%, and the highest survival rate was 31.5%. When the concentration of nystatin in the medium reached 40mg/L, the fungal infection rate decreased significantly, and the highest survival rate was 34.5%. So adding appropriate concentration gradient of antibiotics can reduce the infection rate of explants in the laboratory, so as to improve the survival rate.

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