Experimental Study on Optimization of Culture Medium and Culture Environment of Bacillus Megaterium

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Abstract. The medium components and culture conditions were investigated through orthogonal test. The experiment showed the optimum fermentation medium (g/L) was composed of peptone 10, beef paste 6, yeast extract 6, sodium chloride 7, agar 15,PH=8.0; The suitable culture conditions were as follows: culture temperature was 30°C, rotating speed was 200r/min, liquid level was 60mL/250mL, inoculation volume was 5%. Bring up under the optimal conditions, using tablet coating method, the optimum fermentation time was 48h, the number of viable cells reached the peak 2.52×10⁸cfu/mL, at this time.

1. Introduction
This experiment is based on the optimization of Bacillus megaterium culture medium and culture environment. After determining the optimal medium distribution ratio and optimal culture conditions of Bacillus megaterium, the strain is expanded and cultured, and the cultured Bacillus megaterium is used. The soil samples were subjected to microbial solidification experiments. [1-3] The activity of Bacillus megaterium is stronger than that of Bacillus licheniformis and has a broader application prospect. In the experiment, we used unsaturated purple soil with a water content of 18% as the experimental soil, aiming to have a deeper research and understanding on microbial reinforcement of soil, and promote the development of this soil reinforcement technology in China.

2. Medium composition optimization orthogonal experiment
In order to finalize the composition of the medium most suitable for cell growth, an orthogonal experiment of four factors and three levels of L₉ (3⁴) was selected. The experimental factors are shown in the table below.
Tab. 1 L₀(3⁴) Orthogonal test of culture medium

| LEVEL | FACTOR | A Peptone (g/L) | B Beef cream (g/L) | C Yeast extract (g/L) | D NaCl (g/L) |
|-------|--------|-----------------|-------------------|----------------------|-------------|
| 1     |        | 10              | 3                 | 3                    | 5           |
| 2     |        | 15              | 6                 | 6                    | 7           |
| 3     |        | 20              | 9                 | 9                    | 9           |

In order to finally determine the composition of the medium most suitable for the growth of the strain, the medium optimized orthogonal experiment was performed on the basis of the single factor experiment using four factors and three levels for orthogonal experiment. From the orthogonal experimental results of the culture medium and the visual analysis table, it can be seen that the influence of various medium components on the growth of Bacillus megaterium is beef extract > NaCl > protein > yeast extract, and the result is that the Bacillus megaterium medium is known. The optimum ratio of the ingredients (g/L) is peptone 10, beef extract 6, yeast extract 6, sodium chloride 7. From the orthogonal experimental variance analysis table of the culture medium, it was found that peptone, beef extract, yeast extract, and sodium chloride as components of the culture medium had a significant effect on the growth of Bacillus megaterium.

3. Culture condition optimization orthogonal experiment
In order to determine the optimal culture conditions, the L₀(3⁴) orthogonal experiment was performed with 4 factors and 3 levels. The level and factors of the orthogonal experiment were determined in the following table, and cultured in the optimized medium for 24 hours.

Tab. 2 L₀(3⁴) Orthogonal test of culture conditions

| LEVEL | FACTOR | A Culture temperature (℃) | B Vaccination conditions (% V/V) | C Bottle volume (mL/250ML) | D Shaker speed (r/min) |
|-------|--------|---------------------------|----------------------------------|-----------------------------|------------------------|
| 1     |        | 45                        | 5                                | 100                         | 200                    |
| 2     |        | 30                        | 3                                | 60                          | 200                    |
| 3     |        | 20                        | 1                                | 30                          | 200                    |

In order to better optimize the culture conditions, several factors with a greater degree of influence were selected from the influencing factors to carry out orthogonal optimization experiments. From the orthogonal experimental results of the culture environment and the visual analysis table, the influence degree of each culture condition on Bacillus megaterium was temperature > bottle volume > inoculation amount > shaker rotation speed, and the optimum culture temperature of Bacillus megaterium was 30 °C, the shaker speed is 200r/min, the bottle volume is 60mL/250mL, and the inoculum volume is 5% (V/V). It can be seen from the orthogonal experimental variance analysis table of the culture conditions that the temperature has a significant effect on the growth of Bacillus megaterium, and the amount of the bottle and the inoculation amount are second.

4. After the culture liquid is cultured, its activity is tested.
To test the activity of cultured strains, we chose the conductivity test, which is the ability to detect the hydrolysis of urea by Bacillus megaterium, in mmol/(L•min). Conductivity experiments are mainly carried out in two steps. The first step is to measure the conductivity of a certain concentration of ammonia ions to obtain the relationship between the concentration of ammonia ions and the conductivity. The second step is to three cups of 1 mol/L urea nutrient solution. Different amounts of bacterial liquid are added, and the bacterial liquid is diluted to different multiples. At the same time, the bacterial liquid and the nutrient solution react, and the nutrient solution is hydrolyzed under the
action of the bacterial liquid to generate ammonia ions, and the conductivity of the solution at different times is tested, and then the amount of urea hydrolyzed by the bacterial solution can be calculated from the relationship between the conductivity of the nutrient solution measured by the first step and the ammonia ion. In this experiment, the bacterial liquids of the three beakers were diluted 2 times, 5 times and 10 times respectively. When hydrolyzed, we placed the three beakers containing the solution in a constant temperature water bath at 30 °C to keep the temperature constant to control the activity of the bacteria. Before using the conductivity meter, the probe should be immersed in distilled water, and the normal conductivity of the distilled water should be measured before using the test solution concentration. After each test, rinse the probe with distilled water, clean and cover with a cover. The probe should be placed in a fixed position below the liquid level during the test, and should not be close to the wall or bottom of the beaker to avoid excessive errors.

Using the conductivity values of the diluted two-fold bacterial liquid measured in this experiment to make a graph can clearly reflect the relationship of bacterial activity with time. As shown in Figure 1, B. megaterium activity decays with time, but the tendency to decay gradually slows down and the decay rate is slower. The initial value of bacterial activity was 3.655mmol/(L•min). The attenuation in the first 200 minutes was relatively fast, and the latter trend was slow. Until 10h, the activity of Bacillus megaterium was 1.434mmol/(L•min), still greater than 1.0mmol/(L•min). By 24 h, the bacterial activity was reduced to 0.816 mmol/(L•min), indicating the presence of Bacillus megaterium within 24 h. In the subsequent curing experiment, on the basis of this, a new bacterial liquid is injected into the solidified soil sample every 24 hours, and the bacterial activity can be fully utilized to achieve the desired curing effect.

5. Expanded culture and microscopic morphology

After determining the optimum growth conditions and medium components of Bacillus megaterium by orthogonal experimental results, it is necessary to expand the culture of Bacillus megaterium to meet the needs of curing experiments. Some preparations need to be carried out before expanding the culture. First, a certain amount of liquid medium is prepared, and a plurality of 250 mL conical flasks, a 50 mL measuring cylinder, and an 800 mL glass bottle containing the medium solution are placed in a steam pressure sterilizer for sterilization, and then inoculate to the ultra-clean workbench, dispense the culture solution in the 800mL glass vial into a 250mL Erlenmeyer flask with 50mL graduated cylinder, add 60mL culture solution to each conical flask, and take 3mL (5%) bacterial stock solution with the inoculation gun. Add to the culture solution of each conical flask, cover the plug and put on
the rubber band, and put the prepared conical flask into the incubator for cultivation. Set the parameters to 30 °C, 200r/min, and culture for 48h. 2 is shown. During the course of the experiment, when opening and covering the conical flask and the glass bottle, the outer flame of the alcohol lamp should be used to bake at the mouth of the bottle to kill some residual stubborn bacteria to ensure the accuracy of the experimental results.

After expanding the culture of Bacillus megaterium, in order to further understand its existence under the microscopic state, we will make the slides of the cultured bacteria into a microscope and magnify it by 100 times under the electron microscope, as shown in Fig. 2, which is magnified 100 times and then in the electron. The morphology of Bacillus megacephala seen in the microscope.

Fig. 2 The bacterial morphology after magnifying 100 times

6. Effects of environmental conditions on the growth of Bacillus megaterium
The main external environment affecting the growth of Bacillus megaterium is temperature, pH, shaking speed, etc. The temperature has a significant effect on the growth of the cells. In the appropriate temperature range, the increase in temperature is conducive to the promotion of bacterial metabolism, thus enabling bacterial growth and reproduction is accelerated, but exceeding or falling below the appropriate temperature range can greatly affect bacterial activity. High temperatures can degrade bacterial proteins and even inactivate bacteria, while low temperatures tend to greatly inhibit bacterial growth and reproduction, and sometimes cause bacteria to lose activity.

Bacillus megaterium is a microorganism in the soil, and most of the microorganisms in the soil belong to low-temperature microorganisms. Their optimal growth temperature is 25-35 °C, but they can basically survive in the temperature range of 15-45 °C. The orthogonal experiment results in this experiment showed that when the temperature was 45 °C, the number of bacteria was almost 0 after 48 hours of culture in solid medium. Considering the reason, the solid medium may be too thin, and the water in the solid medium in the incubator is evaporated to cause bacterial death; when the culture temperature is 20 °C, the number of viable cells obtained by culture is also very small, the maximum value is only 0.144×10⁸ cfu/mL; when the culture temperature is 30 °C, the number of viable bacteria obtained by culture is the most, The peak value reached 2.520×10⁸ cfu/mL. From this we can see that the solidified soil with Bacillus megaterium is suitable for the warmer spring and autumn climate, and is not suitable for high temperature or low temperature environment such as summer and winter.

pH is also one of the important factors affecting the metabolism and growth of bacteria. The influence of pH is multi-faceted, which mainly reduces the absorption and utilization of nutrients by bacteria by affecting the charge on the surface of bacterial cell membrane. At the same time, pH can also change the degree of ionization of organic compounds in bacterial culture media, which has an indirect effect on bacteria. Each microorganism usually has an optimum pH for growth, with the
highest bacterial activity and highest growth rate in this pH range. The suitable pH for the growth of Bacillus megaterium is neutral, and the acidity of the pH is not conducive to the growth of bacteria or even bacterial rupture. In the experiment, it is considered that the pH value is difficult to grasp during the experiment, and there are many studies on the optimum pH. Therefore, for the optimum growth pH value of Bacillus megaterium, we determine according to the data provided in the bacterial purchase specification. The optimum pH is 7.0.

7. Conclusion
Through orthogonal experiment, Bacillus megaterium activity test and soil solidification experiment, the optimal medium ratio, optimum culture environment and bacterial activity of Bacillus megaterium were studied: under the optimal conditions for 48h, the number of bacteria lived reached 2.520 x 108 cfu/mL. The activity of Bacillus megaterium gradually decays with time and the decay rate is slower.

References
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