Interview with Prof. Zhang

Q: Hello, Prof. Zhang! We learned that you have published an article on the inhibitory effect of persimmon extract on Bacillus subtilis long ago. Bacillus subtilis is one of the protagonists of our project, so for the cultivation of Bacillus subtilis, could you tell us any points to note compared with the usual culture of E. coli?

A: To cultivate Bacillus subtilis, it is necessary to ensure that the pH value, the redox potential of the culture solution and the environmental temperature are relatively stable within a certain tolerance range.

With the extension of the cultivation time, nutrients are consumed and products are discharged and accumulated, resulting in changes in pH and redox potential. Even due to the effect of bioheat, the temperature may fluctuate relatively greatly. In addition, special attention should be paid to the ratio of available carbon source, aging carbon source and nitrogen source in the medium.

The experiment operation should be carried out strictly in accordance with the guidance of the textbook. In fact, the key lies in the design of the experiment. The design of the experiment needs to be determined repeatedly before the start to experiment, including the consistency of the conditions between the control groups and the repeatability of the experiment.

Q: Our project is designed to co-culture *Cyanobacteria* and *Bacillus subtilis*. May I ask Prof. Zhang how we should determine the culture conditions so that the two strains can be cultivated together?

A: The mixed symbiosis culture of two microorganisms is not simply putting them together, nor is it a matter of pure mixing ratio. First of all, you need to compare the main components of LB medium and *Cyanobacteria* medium (BG11 medium). On the one hand, the types and supply of carbon sources and nitrogen sources; on the other hand, the changes in pH and redox potential after the medium is sterilized.

Then you culture the two strains with a single medium to analyze the factors that affect growth, such as the content of growth factors, changes in pH and redox potential, and even the supply of carbon and nitrogen sources. Next, according to the results of the analysis, improve the composition of the medium and then seed *Bacillus subtilis* and *Cyanobacteria.*

Finally, analyze the generation time of *Cyanobacteria* and Bacillus subtilis to determine the ratio of these two in the co-cultivation. It should be noted that the kind, whose generation time is short, tends to grab resources and inhibit the growth

of other kinds. Therefore, this mixing ratio needs to be explored by orthogonal experiments to finally determine the conditions for symbiosis between them.

Q: Prof. Zhang, we got it. And our current method is to mix LB medium and BG11 medium in different proportions, and then seed the two bacteria. The preliminary result is that both strains have grown.

A: On this basis, you also need to set up a large number of control experiments, using *Cyanobacteria* medium alone to cultivate *Cyanobacteria*, and LB medium alone to cultivate *Bacillus subtilis*, and then cross-culture. Because when the two coexist, *Cyanobacteria* can secrete nutrients needed for the growth of *Bacillus subtilis*.

In addition, the composition of BG11 medium is very simple and cannot be used to cultivate Bacillus subtilis alone.

Q: In the current system, do we need to pay special attention to the amount of inoculation?

A: This is the work of experimental design, which is determined after comparison with the data of orthogonal experiments.

First of all, you need to determine an approximate ratio based on the generation time and biomass of the microorganisms, and appropriately reduce the inoculation amount for the faster-producing strains.

Then design a series of combinations of different ratios of medium and inoculum, and conduct orthogonal experiments. The analysis software can be used in advance to optimize various combinations using response surface analysis. For example, when designing different combinations, the 2:1 medium is only inoculated at a ratio of 1:1 and 2:1, and other ratios of medium are inoculated with other ratios of bacteria. In this way, the number of groups in the experiment is reduced.

Finally, select a combination with the largest growth volume, and analyze the factors that affect its growth, such as the ratio of the medium or the ratio of the inoculum that has a greater impact on it.

In summary, starting from the experimental design, through orthogonal experiments, response surface analysis and other methods, the purpose of reducing the amount of experimental data is achieved. It should be noted that the final optimization results obtained by orthogonal experiments are isolated, but the optimal results of response surface analysis may not appear in these results because it is linear and continuous.

Q: We are currently extracting and identifying extracellular polysaccharides secreted

by Bacillus. Before the experiment, we searched the literature about the extraction scheme and found that there are many ways to record in various documents. Does Mrs. Zhang have any recommended methods to extract polysaccharides efficiently? In addition, what methods are generally used to remove proteins and other small molecules in the process of extracting polysaccharides?

A: The method of water extraction and alcohol precipitation is recommended. First, add alcohol solution to dissolve it, resulting in flocculent precipitation. If the color is darker, you can choose to decolorize. There are various decolorization methods. For example, activated carbon adsorption has the advantage of not destroying the structure of polysaccharides and ensuring stability, but the disadvantage is that the decolorization efficiency is not high; there is also hydrogen peroxide, which has the advantage of high efficiency, but may destroy functional groups.

If you only need to separate the polysaccharides in the end, you can choose the Sevage method to remove the protein by denaturing the protein.

Using different concentrations of ethanol can achieve gradient separation of polysaccharides of different sizes. The lowest ethanol concentration of 30% can be precipitated to obtain the highest quality fragments, 50% ethanol can obtain medium-sized fragments, and 70% or more ethanol can obtain small fragments.

In addition, the separation and purification of polysaccharides can also be achieved by column chromatography.

Q: Our project is to overexpress synthetic polysaccharide genes in Bacillus subtilis. When determining the content of polysaccharides, do we still need to determine the number of bacteria?

A: The amount of inoculation must be consistent, and the OD value can be measured after the growth is over to ensure that the amount of growth is also roughly the same. It can also be weighed directly, and information about the number of bacteria can be obtained through the quality of the culture. Or perform a cell plate count to calculate the bacterial density.

Q: We all know that Bacillus subtilis will form spores to protect itself when subjected to environmental stress. How does this spore participate in the material cycle after it is formed?

A: There are several ways for spores to participate in the material cycle:
First, it is ingested by other animals as food, and is digested and lysed. Of course, it can exist stably in the animals for a long time if the environment is suitable;
Second, the spores that remain in the natural environment for too long and still

have not germinated may disintegrate as their own structure collapses due to the gradual loss of water or the effects of other environmental factors.

Q: Prof. Zhang, what are the main aspects of the influence of water on the growth of microorganisms?

A: First of all, water affects all metabolic processes. Because the proportion of free water is very high, accounting for more than 90% or even 95% of the total water volume, and even some aquatic microorganisms may reach more than 99%. For molds, it may be slightly lower than yeasts, but the free water content can reach 85%.

Secondly, the influence of moisture is also reflected in the vapor pressure of the soil environment.

In addition, water also affects the movement of cells. The chemotactic movement of bacteria is paradoxical. It will spontaneously move to nutrient-rich areas, but osmotic pressure and water resistance will prevent this movement. When the growth status is good, its active movement is dominant and can overcome the resistance of water. Some bacteria have flagella structure, which can strengthen this active movement. Also note that water generally cannot carry spore movement.

Q: Teacher Zhang, what is the exercise capacity of algae microorganisms?

A: The *Nostoc* sp. that you used has no flagella, so it can only perform passive movements. However, there are some species of algae microorganisms that can be very fast. Bacillus subtilis is relatively a common large-scale structure. Air flow and water flow will exert certain force on it, affecting its speed and direction of movement.