Fermentation Optimization of Bacillus Licheniformis

Sui Ming1,2, Yang Zhang3, Yonglin Guan1, Chunming Li1, Xuexiang Shu1, Jiachao Rong3, Rongqing Zhou2, Guoying Li2, *

1Department of wine and food engineering, Si Chuan Technology & Business College, Si chuan, Du jiang-yan 611800, China
2Key Laboratory of Leather Chemistry and Engineering, Ministry of Education and College of Light Industry, Textile & Food Engineering, Sichuan University, Chengdu 610065, China
3Tongren Polytechnic College, GUI ZHOU, Tong ren, 554300, China

*Corresponding author e-mail: guoyingli372021@yeah.net

Abstract. Optimizing the fermentation conditions of Bacillus licheniformis and increasing the yield of Bacillus licheniformis to better serve agriculture is a very important research. The purpose of this experiment was to investigate the optimal fermentation conditions for increasing the production of Bacillus licheniformis. Through a series of investigations on Bacillus licheniformis, the system has learned the optimization method of the culture medium and the analysis method of the experimental data, and strengthened the basic experimental operation skills to lay the foundation for future experimental exploration.

1. Introduction
The research of Bacillus licheniformis is currently a hot topic, and it is widely used in all aspects of production and life because of its unique nature. Bacillus licheniformis can effectively protect the roots of soil crops, thereby reducing the harm of pests and diseases in the soil to crops. The protection of soil crops by Bacillus licheniformis significantly reduces the use of pesticides in agriculture and makes pesticides abused. [1-3] The phenomenon has been significantly improved and has contributed greatly to environmental protection. Therefore, the nutrition of soil crops, especially Bacillus licheniformis, should be fully supplemented to reduce the impact of pests and diseases on soil crops.

2. Growth curve
The experimental results are shown in Fig. 1. The growth of Bacillus licheniformis is slower and slower at 0-11h; the growth of Bacillus licheniformis is faster at 12-18h, which is logarithmic growth phase; 19-24h It is a stable region for the growth of Bacillus licheniformis, followed by a decline period. It should be in the logarithmic growth phase of Bacillus licheniformis, so the seed liquid in the period of 12h-18h is selected for fermentation, and the exploration of the growth curve lays a theoretical foundation for the subsequent exploration experiments.
3. Carbon source optimization

The number of Bacillus licheniformis cultured in fermentation medium with different carbon sources is shown in Table 1:

| Carbon source          | Yield                  |
|------------------------|------------------------|
| Soluble starch (0.5%)  | \((227\pm0.2)\cdot10^8 CFU/ml\) |
| glucose (0.5%)         | \((47\pm0.3)\cdot10^8 CFU/ml\) |
| sucrose (0.5%)         | \((61\pm0.2)\cdot10^8 CFU/ml\) |
| corn starch (0.5%)     | \((148\pm0.5)\cdot10^8 CFU/ml\) |
| Sodium citrate (0.5%)  | \((1\pm0.3)\cdot10^8 CFU/ml\) |

It can be seen from Table 1 that the effects of five carbon sources on Bacillus licheniformis are: soluble starch > corn starch > sucrose > glucose > sodium citrate. By comparison, soluble starch has a strong promotion to the growth of Bacillus licheniformis. Effect; corn starch, glucose and sucrose have a certain promoting effect on the growth of Bacillus licheniformis but not as effective as soluble starch; in the medium with sodium citrate as the carbon source, Bacillus licheniformis hardly grows, indicating lemon sodium has an inhibitory effect on the growth of Bacillus licheniformis. Therefore, it has been found that the optimal carbon source for the fermentation broth of Bacillus licheniformis is soluble starch.

4. Optimization of nitrogen source

The number of Bacillus licheniformis cultured in fermentation medium with different carbon sources is shown in Table 2:

| Nitrogen source          | Yield                  |
|--------------------------|------------------------|
| Beef cream (0.5%)        | \((105\pm0.5)\cdot10^6 CFU/ml\) |
| Peptone (0.5%)           | \((10\pm0.3)\cdot10^6 CFU/ml\) |
| Bean cake powder (0.5%)  | \((2\pm0.3)\cdot10^6 CFU/ml\) |
| NaNO₃ (0.5%)             | \((11\pm0.5)\cdot10^6 CFU/ml\) |
| \((NH₄)₂NO₃\) (0.5%)     | \((8\pm0.3)\cdot10^6 CFU/ml\) |

It can be seen from Table 2 that the effects of five nitrogen sources on Bacillus licheniformis are: beef extract > \((NH₄)₂NO₃\) > peptone > \((NH₄)NO₃\) > bean cake powder. By comparison, it is found that beef extract has growth on Bacillus licheniformis. Significant promotion, while peptone, bean cake powder,
NaNO₃, (NH₄)NO₃ did not promote the growth of Bacillus licheniformis. Based on the above data analysis, the best nitrogen source for promoting the growth of Bacillus licheniformis is beef extract.

5. **Hill climbing experiment**
   According to the climbing experiment, the most suitable concentration range of three significant factors of Bacillus licheniformis fermentation medium was determined. The concentration range of starch, beef extract, NaCl, starch 1.5%, beef extract 1.5%, NaCl 0.1% corresponding fermentation The highest number of spores is the maximum response area of the three factors.

   **Table 3.** Gradient design and results of climbing experiment

   | No. | X_A | X_B | X_C   | Y(×10⁸ cfu·mL⁻¹) |
   |-----|-----|-----|-------|------------------|
   | 1   | 1%  | 1%  | 0.05% | 5.4±0.5          |
   | 2   | 1.5%| 1.5%| 0.1%  | 7.2±0.3          |
   | 3   | 2%  | 2%  | 0.15% | 6.5±0.3          |

6. **Box-Behnken test**
   After determining the optimum concentration of the significant factor, the response surface analysis was carried out with 1.5% starch, 1.5% beef extract and 0.1% NaCl. The experimental design and results are shown in Table 4. The regression equation model fitted by Design expert 8 is:

   \[ Y = 9.56 + 1X_A + 0.63X_B + 0.13X_C + 0.75X_AX_B + 0.25X_AX_C + 1X_BX_C - 1.53X_A^2 - 2.78X_B^2 - 1.78X_C^2 \]

   Equation regression analysis is shown in Table 5.

   **Table 4.** Box-Behnken design factor and level list

   | Factors | -1 (low level) | 0 (center point) | 1 (high level) |
   |---------|----------------|------------------|----------------|
   | X_A     | 1%             | 1.5%             | 2%             |
   | X_B     | 1%             | 1.5%             | 2%             |
   | X_C     | 0.05%          | 0.1%             | 0.15%          |

   **Table 5.** Analysis of variance of regression model

   | source          | Seq SS | DF | Adj SS | F-value | P-value |
   |-----------------|--------|----|--------|---------|---------|
   | Model           | 79.43  | 9  | 8.83   | 12.81   | 0.0014  |
   | A-A starch      | 8      | 1  | 11.61  | 0.0113  |         |
   | B-B Beef cream  | 3.13   | 1  | 4.54   | 0.0707  |         |
   | C-C NaCl        | 0.13   | 1  | 0.18   | 0.6829  |         |
   | AB              | 2.25   | 1  | 3.27   | 0.1137  |         |
   | AC              | 0.25   | 1  | 0.36   | 0.5659  |         |
   | BC              | 4      | 1  | 5.81   | 0.0468  |         |
   | A²              | 9.86   | 1  | 14.31  | 0.0069  |         |
   | B²              | 32.54  | 1  | 47.24  | 0.0002  |         |
   | C²              | 13.34  | 1  | 19.37  | 0.0032  |         |
   | Residual        | 4.82   | 7  | 0.69   |         |         |
   | Lack of fit     | 3.7    | 3  | 1.25   | 4.66    | .0855   |
   | Pure Error      | 1.07   | 4  | 0.27   |         |         |
   | Total           | 84.25  | 16 |        |         |         |

   From the analysis of variance, P=0.0014 indicates that the regression equation is very significant, and R²=94.28%, indicating that the response model can explain the change of fermentation level of 94.28%, which can reflect the fermentation process of Bacillus licheniformis.
Observing the trend of the above figures, it can be seen that $X_A$, $X_B$, and $X_B$ have maximum values in the test range. The optimal test points for these three components were $(0.3, 0.2, 0.1)$, i.e. starch $16.5$ g/L, beef extract $16.9$ g/L, NaCl $10.5$ g/L, the predicted fermentation spore concentration at this point was $9.6 \times 10^8$ cfu/mL. It can be seen that the $X_AX_B$ and $X_BXC$ contour lines are elliptical, the interaction is significant, and the $X_AXC$ contour is approximately circular, and the interaction is not significant.

7. Conclusion

Through the three sets of parallel experiments, the following conclusions were drawn: the single factor experiment concluded that the optimal carbon source for the fermentation medium of Bacillus licheniformis was $5$ g/L soluble starch, and the best nitrogen source was $5$ g/L beef paste. Plackett-Burman experiment concluded that the three significant influencing factors were optimized, and the optimum number of spores was best when starch $16.5$ g/L, beef extract $16.9$ g/L, NaCl $10.5$ g/L. After optimization, the number of spores of Bacillus licheniformis reached $9.6 \times 10^8$ cfu/mL, which was 3.7 times higher than the number of spores before optimization ($2.6 \times 10^8$ cfu/mL). The experimental results were obvious.

References

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