Screening of Cellulose Degrading Bacteria and Construction of Complex Microflora

Ming Sui¹ *, Jiachao Rong², Yang Zhang², Huirong Xie¹, Cai Zhang¹

¹Department of Wine and Food Engineering, Sichuan Technology & Business College, Dujiangyan, Sichuan Province, China; ²Department of economics and management, Tongren Polytechnic College, Gui Zhou, Tongren, 554300, China

*Corresponding author email: mingsui@stbu.edu.cn

Abstract. The collected samples were pre-screened with Congo red cellulose medium according to the ratio of transparent circle diameter/colony diameter, and 10 strains with strong ability to decompose cellulose were obtained. Then, these strains were inoculated into the liquid production enzyme medium to measure the CMC enzyme activity for rescreening, and finally the N05, N13, N213 strain high-efficiency cellulose degradation strains were screened. These three strains were combined and cultured, and the best combination of effects was obtained by comparing the enzyme activities.

Keywords: material, biology, bacteria.

1. Introduction
The purpose of this study is to screen high-efficiency cellulose-degrading bacteria to combine them into a composite flora, and to determine the cellulose degradation effect of the composite flora in order to solve the problem of full utilization of straw resources. Separation and purification of cellulose-decomposing bacteria are usually carried out using a filter paper refining tube. The test is sampled from rotted cotton stalks, bovine rumen fluid and forest dead wood, and is purified by concentration gradient dilution method and repeated scribing method, and Congo red-carboxyl Cellulose-degrading bacteria were screened on the cellulose-based medium plate. The cellulase activity and the corn stalk degradation rate in the fermentation broth were determined to determine the ability of the strain and the mixed bacteria to degrade cellulose. Finally, the screening strains were identified. Through this study, it provides a new way and theoretical basis for the development and utilization of straw feed resources.

2. Test reagent
Sampling: Separation of samples from rotted cotton rods, cattle rumen fluid from slaughterhouses, forest dead wood and soil. The soil at the surface of 5 cm to 15 cm was collected by the surface soil, and the soil was collected by a four-point method and stored in a sterile bag at 4 °C. Corn stalk sample: taken from the experimental station of Shihezi University, washed, dried, pulverized,
pretreated with 1% dilute sulfuric acid for 2 hours at room temperature, washed to neutral, dried, passed through a 40 mesh sieve, and set aside.

DNS reagent: Accurately weigh 5 g of 5,5-dinitrosalicylic acid dissolved in distilled water, add 10 g of sodium hydroxide, 100 g of sodium potassium tartrate and 250 ml of water, and then add 1 g of crystalline phenol after heating and dissolving. 0.5 g anhydrous sodium sulfite, after all dissolved, cool, dilute to 500 ml volumetric flask with distilled water, store in a brown bottle for one week before use, and filter before use.

0.05 mol/L, pH 4.8 citrate buffer: Weigh 4.83 g of citric acid monohydrate, dissolve in about 750 ml of water, add 7.94 g of trisodium citrate during stirring, and make up to 1 000 mL with distilled water. In the volumetric flask, adjust the pH of the solution to ((4.80±0.05) for use. 1% CMC-Na solution: Weigh 2.0 g of sodium carboxymethylcellulose and dissolve in 150 ml of lemon Acid buffer and heat to 80°C to 90°C, stir while heating until all sodium carboxymethyl cellulose is dissolved, after cooling, dilute to 190 mL with buffer, then adjust the solution with 2.0 mol / L hydrochloric acid or sodium hydroxide The pH was adjusted to 4.80±0.05, uniformly stirred to a 200 mL volumetric flask, and finally stored in the refrigerator for later use.

3. Screening of cellulose degrading bacteria

Weigh 1 g of the enriched culture into a 150 ml flask containing 99 ml of sterile water, shake it on a constant temperature shaker for 45 minutes at 30 °C, then let stand for 30 minutes, and take the supernatant for gradient. Dilute 10 times, repeat each dilution 3 times, and incubate at 30 °C. Repeated scribing to separate the bacteria to grow out and purify into a single colony.

The isolated and purified single bacteria were inoculated on a cellulose agar plate, cultured in a 30 °C incubator for 3 days, and the colonies on the plate were stained with a 1 mg/mL Congo red solution for 30 min, and washed with distilled water. Finally, decolorization was carried out with a 1 mol/L NaCl solution for 30 minutes. The diameter of the transparent circle and the colony was measured. The primary screening standard was the diameter of the transparent circle/colony diameter. The strain with the larger ratio was inoculated on the slant medium and stored at 4 °C.

The strain producing the obvious hydrolyzed circle was inoculated into the liquid medium under the condition of 30 °C and 120 r/min, and the seed was obtained after the culture for 3 days, and then inoculated into the liquid by the 2% inoculation amount under the same culture condition. In the enzyme-producing medium, the CMC is measured every 24 hours, and the method for measuring the enzyme activity is referred to the light industry standard in China.

Extraction of crude enzyme solution: The culture solution was centrifuged at 3000 r/min for 15 minutes, and the supernatant was taken as a crude enzyme solution.

(A). Drawing of the glucose standard curve

Wash and dry 10 ml plugged test tube 6 pieces, add distilled water and standard glucose solution after numbering, and prepare a series of different concentrations of glucose solution, as shown in Table 2-1. Add 3 ml of DNS reagent to each tube while shaking well, shake again, then boil in water for 5 minutes, take out and cool to room temperature, use No. 0 tube as blank control at 540 nm wavelength, adjust the zero point to measure each tube solution. The optical density value and record the result. The glucose standard curve is drawn by taking the corresponding optical density value as the ordinate and the glucose content as the abscissa.

| Number | 0 | 1 | 2 | 3 | 4 | 5 | 6 |
|--------|---|---|---|---|---|---|---|
| Distilled water | 2.0 | 1.6 | 1.4 | 1.2 | 1.0 | 0.8 | 0.6 |
| Glucose standard solution | 0 | 0.4 | 0.6 | 0.8 | 1.0 | 1.2 | 1.4 |
| Sugar content | 0 | 0.4 | 0.6 | 0.8 | 1.0 | 1.2 | 1.4 |

(B). Determination of cellulase activity
Using DNS color method, take 10 ml of washed and dried test tube, after the number is preheated for 5 to 10 minutes in a 50 °C water bath, then add CMC-Na buffer solution 2.0 ml and 0.5 to each tube. Mix the ml enzyme solution, keep it for 30 min in a 50 °C water bath, add 3 ml of DNS reagent immediately after removal to stop the enzyme reaction, heat for 5 min in a boiling water bath, remove and cool to room temperature and dilute to 10 ml with distilled water. The optical density value of the blank control and the treatment enzyme solution was measured at a wavelength of 540 nm, and the amount of glucose contained in the enzyme solution was determined based on the glucose standard curve, thereby calculating the enzyme activity.

After several generations of separation and purification, there were 23 strains of hydrolyzed circles on Congo red cellulose agar plates, and their hydrolysis patterns on the plates. After 4 days of culture, the diameter of the transparent circle/colony diameter was greater than 1, and 10 strains with good decomposition were initially screened. The growth of each strain on the Congo red cellulose agar plate is shown in Table 2.

**Table 2. Primary screening results of cellulose degrading bacteria**

| Number | Transparent ring diameter A | Colony diameter B | A/B |
|--------|-----------------------------|------------------|-----|
| N03    | 15                          | 10               | 1.5 |
| N04    | 17                          | 11               | 1.6 |
| N05    | 21                          | 10               | 2.1 |
| N06    | 14                          | 11               | 1.3 |
| N08    | 16                          | 12               | 1.4 |
| N11    | 18                          | 13               | 1.4 |
| N13    | 20                          | 10               | 2.0 |
| N16    | 15                          | 9                | 1.7 |
| N17    | 18                          | 10               | 1.8 |
| N21    | 19                          | 9                | 2.1 |

Studies have shown that the strains degrading Visa have a clear hydrolyzed circle on the Congo red, and the level of enzyme activity is positively correlated with the size of the hydrolyzed circle. It has also been confirmed that the ratio of the transparent circle to the diameter of the colony can reflect the enzyme activity of the strain. High and low. It can be seen from Table 2 that the colony diameter ratios of the 10 strains are different, and the hydrolyzed circle of the strain with large ratio is correspondingly large, indicating that its enzyme activity is higher than that of other strains. It is found that the difference in the absorption of nutrients and the metabolites on the plate and in the liquid, medium will affect the size of the hydrolysis zone. The factors affecting the size of the hydrolysis zone are also the growth degree of different strains, the rate of enzyme production, and the cellulase production. The difference will also have an effect; the structural characteristics of bacterial cell walls and fungi will also have an effect on the production of enzymes; the factors affecting the size of the hydrolysis zone also include the amount of screening bacteria and the thickness and type of the medium, such as some strains in the methyl group The CMC enzyme secreted on the cellulose sodium medium is more, so that its hydrolysis cycle is larger. All of the above reasons will cause the hydrolyzed circle to be inconsistent with the enzyme activity, so it is essential to measure the enzyme activity in liquid fermentation. In summary, the method for preliminary quantification of the enzyme production of the primary screening strain is to observe the size of the hydrolysis zone and determine the ratio of colony to transparent circle diameter, but it cannot be used as the only quantitative indicator in terms of enzyme production activity. Plate screening will be affected by various factors, so it can not reflect the ability of the strain to produce cellulase very accurately. However, by observing the size of the transparent circle on the plate by preliminary screening, the enzyme production of some strains can be eliminated, greatly improving the obtained. The probability of efficient strains. Therefore, in order to screen out better high-yield strains, it is necessary to rely on liquid fermentation...
to measure enzyme activity and then rescreen. Determination of the CMC enzyme activity of the liquid fermentation crude enzyme solution is a criterion for screening high-yield cellulase strains.

In the rescreening step, the strain was tested for 6 consecutive days to select a high-yield cellulose strain. By doing the same record table, it can be seen from the results that the strains N05, N13, and N21 have the highest CMC activity, so it is determined that N05, N13, and N21 are high-efficiency strains. Comparing the CMC enzyme activities of each strain, it can be seen that the CMC enzyme activities of different strains are significantly different and increase or decrease with time. The cellulase activity may be affected during the cellulase activity, and the cellulase activity may be affected. The structure of the cellulose substrate has an amorphous region and a crystalline region, and the amorphous region is easily decomposed, but the crystalline region It is difficult to be decomposed; cellulase is composed of complex enzymes. Different culture environments and different strains will make the enzyme system very different, so the process of decomposing cellulose will be very Large differences, which in turn make it difficult to measure the activity of a single component in a crude enzyme solution; and also have different ability to decompose cellulose due to different strains. Studies have shown that only by the synergistic mutual aid, various enzyme systems can completely decompose the natural cellulose of straw, so the single strain is not capable of decomposing the natural cellulose of straw under normal conditions. Therefore, in actual production and application, only the synergistic compounding of microorganisms can improve the ability of the strain to decompose the natural cellulose of straw.

4. Construction of complex bacteria

The selected dominant strains were transferred from the slant medium to the liquid medium. After the expansion, 0.5 mL of the plate was applied to the carboxymethylcellulose plate medium, and other strains were planted on the surface of the medium plate, and cultured at 30 ° C for 2 times. Up to 5 days, it was observed whether there was antagonism or growth inhibition between the grown strains. The strains obtained by the rescreening were combined in a volume ratio of 1:1, and they were inoculated into a liquid enzyme-producing medium under the culture conditions of 30 ° C and 120 r/min, respectively, to measure the enzyme activity of the CMC of the culture. A good combination of flora was determined by measuring the size of their enzyme activity at intervals of 6 days every 24 hours. The cellulase activity of the flora was also determined by the dinitrosalicylic acid colorimetric method.

The cellulose strain with strong degradation ability was isolated, purified and screened into a suspension of the bacteria, and the inoculum amount was inoculated into the liquid production enzyme medium at a volume ratio of 1:1, at 30 ° C, 120 r / min culture conditions, each The enzyme activity was measured for 24 hours, and finally the composite strain SDP having strong ability to decompose cellulose was selected from the samples, and the activity of each combined enzyme was tested for comparison. The results are shown in Table 3.

Table 3. Results of enzyme activity determination of each combined strain

| Number       | Days |   |   |   |   |   |
|--------------|------|---|---|---|---|---|
|              | 1    | 2 | 3 | 4 | 5 | 6 |
| N05          | 1.97 | 1.95 | 2.06 | 2.37 | 3.56 | 1.73 |
| N13          | 1.15 | 2.02 | 2.86 | 3.07 | 3.66 | 2.02 |
| N21          | 1.23 | 1.89 | 2.35 | 2.66 | 3.67 | 1.97 |
| N05+N13      | 1.78 | 2.07 | 2.09 | 3.05 | 3.25 | 1.87 |
| N05+N21      | 2.13 | 2.44 | 2.86 | 3.25 | 3.73 | 2.13 |
| N13+N21      | 1.87 | 2.35 | 2.77 | 3.06 | 3.33 | 1.96 |
| N05+N13+N21  | 3.45 | 4.43 | 5.12 | 5.57 | 6.07 | 4.78 |

From the results, it was found that the enzyme activity reached the highest on the fifth day. Since the cellulolytic enzyme is a complex enzyme system composed of multiple components, the combined
culture can increase the enzyme activity to some extent, and the SDP (N05+N13+N21) combination has the most obvious effect. According to the theoretical study of niche, it is confirmed that different strains can coexist with each other because they avoid synergy between the populations through synergy. Therefore, the compatibility between the strains provides synergistic combination of strains to degrade natural cellulose. The foundation. The CMC enzyme activity of the compound strain SDP degrading natural straw was better than that of Zeng Qinglan's CMC enzyme activity (1.7 IU/mL), which was also higher than the CMC enzyme activity of the mixed bacteria from the soil. It can also be seen from the table that the enzyme activity of some combinations is lower than that of the single strain, which may be caused by the mutual antagonism of the strains, or the mutual inhibition of the mutual position or secretion between the colonies.

5. Conclusions
In this experiment, CMC enzyme activity was used as an evaluation index to screen out the degrading cellulose bacteria with high activity of N05, N13 and N213 strains, and combined culture was carried out to obtain a better combination. The CMC enzyme activity of the combination was effectively improved. Cui Zongjun and Li Meidan separated and selected four groups of mixed bacteria with high ability to decompose cellulose from the collected samples, and recombined a group of cellulose complex degrading strains with good stability and strong degradation ability based on the principle of acid-base complementation. Lu Yuexia, Lu Zhiwei and other four strains with strong ability to degrade cellulose were separated from compost, rot straw and forest soil. After mixed culture, it was found that the cellulase activity of the mixed culture of single bacteria culture was greatly improved. After 72 hours of culture, the enzyme activity of the composite bacteria can reach 67.12 U, which is equivalent to twice the single strain culture. Feng Wei, Yan Yuhang et al. screened 5 strains of highly active cellulose-degrading bacteria from the soil, and combined them to obtain a combination. The CMC enzyme activity of the mixed bacteria was 3.18 U/ml, which was more than the enzyme activity of the single strain. has seen an increase. Zeng Qinglan selected three strains with good cellulose degradation ability from garbage, soil and livestock manure. After combining the strains, it was found that the degradation ability of any single strain was lower than that of the mixed bacteria, and the CMC activity of the single strain was improved. 1.5 times. The CMC enzyme activity of the composite strain degrading natural straw was better than that of Zeng Qinglan's CMC enzyme activity (1.72 IU/mL). However, with the increase of time, the enzyme consumption of microorganisms can be reduced, and the enzyme activity is gradually reduced. This is similar to the degradation effect of high-yield lignocellulolytic strains studied by Guo Hongwei et al. on corn stover. Since the combination was obtained at room temperature, the strains N05, N13, and N21 were obtained by separation and purification, and the stability after the combination, the relationship between the strains, and the optimum fermentation conditions were yet to be further studied.

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