Study on the culture of Escherichia coli with different hydrolysis depth of globin

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Abstract: Objective: To explore the effect of culture medium made of peptide fragments of different sizes of globin hydrolyzed by protease on the culture of Escherichia coli.

Method: with fresh pig blood as raw material, hydrolyze pig blood protein under fixed conditions, and screen out the enzyme with the highest degree of hydrolysis. Then conduct orthogonal test on the enzyme to determine the best hydrolysis conditions; the beads were obtained under the best hydrolysis conditions. The Escherichia coli was cultured on different peptone culture bases that were prepared by hydrolysate going through different pore size ultrafiltration membrane samples. The culture of Escherichia coli at different hydrolysis depths is determined by detecting the total amount with Escherichia coli detection method.

Results: Trypsin has the best hydrolysis effect. The best hydrolysis conditions are as follows: enzyme dosage of 0.2%, hydrolysis time of 12h, temperature of 50℃, initial pH of 7.5 and mass fraction of substrate protein of 8%. Through growth curves of Escherichia coli, it is known that the smaller the pore size of the ultrafiltration membrane, the smaller the molecular weight of the peptide fragment, and the greater the density of the Escherichia coli bacterial solution.

Conclusion: Through analyzing the production curves of Escherichia coli in different culture medium, it is concluded that the smaller the molecular weight of the protein peptides, the higher the density of Escherichia coli solution in the LB liquid medium prepared by it, and the better the Escherichia coli culture effect.

1 Introduction

In the processing and utilization of pig blood, except that a small part is used for food addition, most of it is discharged as waste, which not only pollutes the environment, but also wastes protein resources[1]. Therefore, we need to make use of pig blood, especially fresh pig blood. It can be decomposed into globin and other substances, and then made into polypeptides needed in the food and pharmaceutical industries through hydrolysis reaction. Polypeptides can form a transparent powder of after being concentrated and dried[2,3]. The peptone contains a lot of amino acids, and more vitamins and other growth factors, which are extremely beneficial to the growth of bacteria such as Escherichia coli and can be used to provide basic raw materials, nitrogen sources, vitamins and other nutrients for the culture of it, which not only improves the utilization rate of pig blood resources, but also accelerate the progress of Escherichia coli culture research[4-6].

2 Test materials and methods

2.1.Materials and instruments

Fresh pig blood (specified pig slaughterhouse in Hefei Economic and Technological Development Zone); trypsin, flavor zyme, neutral protease (Beijing Ruida Henghui Technology Development Co., Ltd.); electric-heating thermostatic water bath HWS28, drying oven DHG-9240A (Shanghai Yiheng Science Instrument Co., Ltd.); Magnetic stirrer SH-3 (Beijing Nuocheng Jiaxin Instrument Co., Ltd.) Constant temperature shaker HASUC (Shanghai Instrument Manufacturing Co., Ltd.); Spectrophotometer V-1100D (Shanghai Meipuda Instrument Co., Ltd.).

2.2.Method

2.2.1.Initial screening of protease

Design different proteases to hydrolyze pig blood separately. Set the mass fraction of substrate protein in the solution as 8%, and hydrolyze them for 6 hours with the optimal temperature pH value. Use formaldehyde titration to measure the amino acid content of the hydrolyzed supernatant.

(1) Take 200 mL of formaldehyde and put it in a beaker. Use NaOH standard solution to adjust the pH to 8.2 during electromagnetic stirring.

(2) Put 5 mL of the solution to be tested in a beaker, and add 60 mL of distilled water to boil, degas and cool. Then adjust the pH to 8.2 with 0.01 mol/L NaOH, and retain for 30 seconds.

(3) Add 20mL of the prepared formaldehyde solution in a slow way and use NaOH standard solution to measure the pH of the solution to 9.2 after a magnetic

stirring for 3 minutes, then record the volume of NaOH consumed V_1; use distilled water as a blank control and record the volume of NaOH consumed V_2. 1000×C× (V_1 －V_2)

(4) Amino acid content of the liquid to be tested

Amino content=200×C×(V_1 － V_2); C: the concentration of NaOH used in the measurement

$$DH/\% = \frac{\text{The amino acid in the Hydrolyzed supernatant}}{\text{Total nitrogen in the sample before hydrolysis}} * 100\%$$

2.2.2.Study on the best conditions for trypsin hydrolysis of globin

The globin is hydrolyzed by single factor control of hydrolysis time, hydrolysis temperature, enzyme dosage, initial pH value and other variables, and then obtain the optimal hydrolysis conditions for trypsin hydrolysis of globin by orthogonal test, as shown in Table 1.

Table 1 Factor level table

	A（Enzyme dosage%）	B（h）	C（℃）	D/PH
1	0.2	11	45	7.5
2	0.3	12	50	8.0
3	0.3	13	55	8.5

2.2.3.Study on the preparation of different peptide filtrates

Add 0.35% sodium citrate solution into fresh pig blood, and remove the serum by centrifugation. Add water of the same volume as red blood cells, then stir and mix the hemolysis to adjust the protein concentration of the reaction solution to 8.0%, and start hydrolysis under the above optimal hydrolysis conditions. After the reaction has stopped, heat and boil for another 10 minutes, then adjust the pH to 5.0, and take the supernatant for use after centrifugation.

Take an equal amount of the homogenized globin hydrolysate and put them through ultrafiltration membrane samples with different pore sizes of 5000Da, 10000Da, 20000Da, 1nm, and 10nm, and detect the free amino acid content and dry matter content in the filtrate.

2.2.3.1.Measurement of free amino acid content

Take 12.5ml of the filtrate sample and put it into a volumetric flask of 250ml. Make a uniform mixing, then draw 20.0ml of the diluted filtrate and put it into a beaker of 250ml and add 60ml of water to re-dilute. Insert the glass electrode and mercury electrode, then turn on the magnetic stirrer, and titrate the solution in the beaker with NaOH standard volumetric solution until the pH value is 9.2. Then add 10.0ml formaldehyde solution, and titrate it to the acidity PH value of 9.2 with NaOH standard volumetric solution. Record the volume of the solution as V1at the moment.

In addition, take 80ml water and adjust the pH to 8.2 with NaOH solution. Titrate it with the above steps and take down the volume of the sodium hydroxide standard solution consumed as V2 for the reagent blank control test.

Calculation

$$X = \frac{(V_2 - V_1) \times C_1 \times 0.014}{V_3 \times \frac{12.5}{250}}$$

In which, X — the content of amino nitrogen in the filtrate;

V1—the volume of NaOH standard volumetric solution consumed after adding formaldehyde into the sample diluent for measurement, ml;

V2—the volume of NaOH standard volumetric solution consumed in reagent blank test after adding formaldehyde, ml;

V3—the amount of sample diluent taken, ml;

C1—the concentration of NaOH standard volumetric solution;

0.014—the mass of nitrogen equivalent to 1.00ml NaOH standard volumetric solution, g;

2.2.3.2.Measurement method of dry matter content

(1) Put the glassware in the oven. Heat it at 100°C for 4h, then take it out and put it in a drying oven for cooling. Weigh it after cooling for half an hour, which is m1.

(2) Weigh a certain amount of filtrate in a glassware. Now the total weight of the glassware and the sample to be tested is m2, then put it in an oven and heat it at 100°C. Taking it out 4 hours later and put it in a drying oven to cool for half an hour, then weigh and mark it as m3.

(3) Calculation: DM=(m3-m1)/(m2-m1)

2.2.4.Study on culture of Escherichia coli with different peptone media

With single factor experiment, calculate the dosage through dry matter. Keep other ingredients unchanged and change peptone. Prepare different medium to cultivate Escherichia coli. Use a spectrophotometer to accurately measure the cell density at 600nm wavelength to compare curves of the culture of Escherichia coli in them. Use the cell density value of bacterial liquid to compare the effect of hydrolysis depth (different peptone) on Escherichia coli culture.

2.2.4.1.Methods of preparation of different peptones

Pass the hydrolyzed globin solution through ultrafiltration membrane samples of 5000Da, 10000Da,

20000Da, 1nm and10nm. Take filtrates that penetrates the membrane for use, and record them as filtrate A, B, C, D, E; pass the five filtrates through reverse osmosis membrane separately, desalting, concentrating and spray-drying so that light yellow transparent powder can be obtained, which are respectively peptone A, B, C, D, E. With single factor experiment, prepare five groups of culture medium. With other components in the culture medium unchanged, add peptone A, B, C, D, E to each medium respectively; five groups of different LB liquid mediums are obtained, which are LB liquid medium A, B, C, D, E for use.

2.2.4.2.Method for preparing Escherichia coli culture medium

(1) Centrifuge the liquid culture medium of the bacteria, then add an equal amount of 40% glycerol to the pellet, and freeze it at 80°C below zero.

(2) Preparation of solid medium

LB solid medium formula: peptone 10g/L yeast extract 5g/L NaCl solid 5g water 1000ml agar 15-20g

(3) Preparation of plates

Take 10.0g of peptone, 5.0g of yeast powder, 10.0g of NaCl, and 800ml of water to dissolve it for the second time, and stir it with a glass rod. Adjust the pH to about 7.4 with 1mol/L NaOH solution, dilute it to 1L, and adjust its pH to 7.4. Pour the above solutions into different conical flasks, about 10cm above the bottom of the flask, and then add agar of 2%. Use a high-pressure steam sterilizer to sterilize them at 120°C for 15 minutes, then take out the culture medium and put them into an electric blast dryer to dry them at 60°C. Pour them into the culture dishes with each containing about 10ml. Conduct plate application on the aseptic operating table after condense.

(4) Inoculate Escherichia coli

Take the Escherichia coli BL21 cryopreservation solution prepared for the experiment, and burn the tube opening with an alcohol lamp, then open the centrifuge tube; put 100 ml of the solution on the plate with a pipette, and then conduct plate application with a sterilized smear stick drawing cross.

(5) Culture of Escherichia coli

Put the inoculated plate upside down in a thermostatic incubator at 37°C, and cultivate for 20 hours to grow colonies. Carry out the inoculation in a freshly sterilized LB liquid medium at 37° C with 229 rpm/min in thermostatic shaking for 10 hours. Then cultivate Escherichia coli in LB liquid medium A, B, C, D, and E respectively.

2.2.4.3. Method for measurement of cell density of Escherichia coli bacteria solution

Take the above-mentioned culture medium and number them as cell suspension A, B, C, D and E; put them into sterile test tubes numbered A, B, C, D, E respectively, and measure their absorbance value at wavelength of 600nm; after shaking up the above cell suspensions at different concentrations, measure their OD values in a cuvette of 1cm with wavelength of 600nm. In the colorimetric measurement, the culture medium without added bacteria can be used as a blank control to measure the OD value of 5 cell suspensions. Compare the growth of thermostatic in five different media with growth curves.

3 Results and analysis

3.1.Analysis of the results of protease screening

With the degree of hydrolysis as an indicator, calculate the hydrolysis degrees of above four enzymes (flavor protease A, trypsin B, compound protease C, and neutral protease D), as shown in Table 2.

Table 2 Hydrolysis degree table of different enzymes

Enzyme type	A	B	C	D
DH/%	27.85	39.80	18.05	23.40

According to the table, among the four enzymes, the degree of hydrolysis is that flavor protease A (27.85), trypsin B (39.80), compound protease C (18.05), and neutral protease D (23.40). The highest degree of hydrolysis is trypsin, that is, Trypsin is selected as the suitable enzyme.

3.2.Analysis of orthogonal experiment results

Table 3 Orthogonal experiment table

	A（%）	B（h）	C（℃）	D/PH	%
1	0.2	11	45	7.5	41.20
2	0.2	12	50	8.0	50.33
3	0.2	13	55	8.5	43.25
4	0.3	11	50	5.5	38.50
5	0.3	12	55	7.5	51.02
6	0.3	13	45	8.0	39.38
7	0.4	11	55	8.0	35.65
8	0.4	12	45	8.5	37.45
9	0.4	13	50	7.5	55.10
K1	44.93	38.45	39.34	49.11	——
K2	42.97	46.27	47.98	41.79	——
K3	42.73	45.91	43.31	39.73	——
R	2.2	7.82	4.67	9.38	——

According to the analysis of orthogonal table, obtain the

best hydrolysis conditions for trypsin hydrolysis of globin, as shown in Table 3.

According to the range value of R, it is known that the primary and secondary order is D>B>C>A, and the order of influencing factors is initial pH>hydrolysis time>temperature>enzyme dosage; the optimal order is A1B2C2D1, and the optimal hydrolysis conditions are: initial pH of 7.5, hydrolysis time of 12h, hydrolysis temperature of 50℃, enzyme dosage of 0.2%.

3.3.esult analysis of Escherichia coli growth curves

The growth effect of Escherichia coli can be compared with the cell density value of the bacterial solution. The figure shows growth curves of Escherichia coli in different culture media. Escherichia coli cell suspension (A, B, C, D, E)

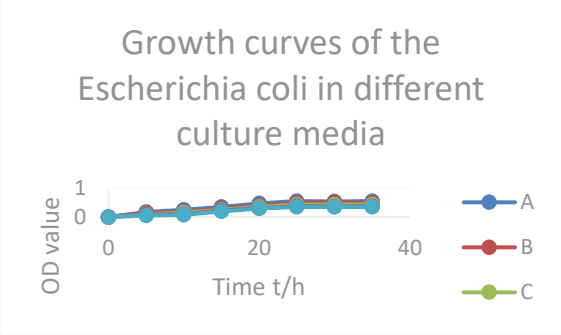

Figure 1 Growth curve of Escherichia coli in different culture media

From the OD value of Escherichia coli cell suspension (A, B, C, D, E), the growth of Escherichia coli in different media is clear to see. The excellent culture effect of Escherichia coli cell suspension is A>B>C> D>E; the smaller the pore size of the ultrafiltration membrane, the smaller the molecular weight of the peptide, and the greater the density of Escherichia coli bacteria solution, that is, the better the growth of Escherichia coli.

4 Conclusion

The experiment results show that pancreatin has the best hydrolysis effect as the hydrolytic enzyme. With single factor and orthogonal experiments, it is obtained that the optimal hydrolysis conditions are: initial pH of 7.5, hydrolysis time of 12h, hydrolysis temperature of 50℃, enzyme dosage of 0.2%, and mass fraction of substrate protein of 8%. Hydrolyze globin under the optimal process conditions, and prepare protein peptide solutions of different molecular weights through ultrafiltration membrane samples of different pore sizes, then calculate the dosage through dry matter, and cultivate Escherichia coli in different LB liquid media with other ingredients unchanged. Finally, accurately measure the density of E. coli cells at a wavelength of 600 nm with a spectrophotometer to compare the effects of Escherichia coli culture. From the growth curves of Escherichia coli in different media, it is concluded through analysis that the smaller the molecular weight of protein peptides, the greater the density of Escherichia coli cultured in LB liquid medium prepared from it, that is, the better the effect of Escherichia coli culture.

Acknowledgements

This work was supported byNatural Science Research Foundation of the Department of Education of Anhui Province (No.KJ2018A0583，KJ2019A0874),
Scientific research team of Anui Xinhuauniversity（kytd201908 ），
Quality engineering project of Anhui Xinhua University（2019xqjdx03 ，2019jxjy45），
University level scientific research project of Anhui Xinhua University（2017zr010）.

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