Experimental Study on Western Blot

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ABSTRACT: In this paper, the expression of specific proteins in two groups of cells was studied by the methods of documentary review and experiment. The results were as follows: one group of cells expressed proteins, and the other group did not.

1. Research Objects and Methods

1.1 Research Object
The expression of specific proteins in the two groups of treated cells was studied as the object.[1-2]

1.2 Methods

1.2.1 Documentary
Currently, the most commonly used methods of insect protein extraction are alkali extraction (Zhao Chenxia et al., 2013, enzyme formulation (Wang Yan, 2012, salt formulation, 2012) and water extraction (buffer solution extraction, 2011) methods. Among them, alkali extraction and enzyme extraction with high protein extraction rate are the most common. At home and abroad, the research on black water gadfly is mainly focused on the treatment of kitchen waste and livestock manure, the production of organic fertilizer and fishery and animal husbandry feed, and the assessment of environmental safety (Newton et al., 2005). The black water gadfly is mainly focused on the treatment of kitchen waste and livestock manure, the production of organic fertilizer and fishery and animal husbandry feed (Diener et al., 2009; Yu Guohui and others, 2009), Biodiesel has also been Li et al., 2011a, by some scholars in recent years (2011) and antimicrobial substances (Erickson et al., 2004; and Zheng et al., 2012; Choi et al., 2012; Summer, 2013), And made preliminary progress.

Searching and consulting literature and research results with the keywords of "Western blot" and "Protein Expression" on CnKI (China National Knowledge Internet), and self-learning the book "Biochemistry and Molecular Biology" and other books related to biochemistry, providing important theoretical basis for the study of this paper.[3-5]

1.2.2 Experimentation
After visiting and learning western blot experiments conducted by professionals, specific proteins extracted from two groups of cells were subjected to western blot experiments.

2. The Experiment Design

2.1 Experimental Grouping Condition
This paper mainly adopts the experimental method of comparing the protein extracted from the
experimental group and control group. Before the experiment began, the two groups of cells were extracted from the tissues of the subjects for comparison. After the two groups of cells were cultured to extract the protein, the experiment began.

2.2 Experimental Materials and Reagents

2.2.1 Reagent

2.2.1.1 PBS (0.01M pH 7.2 ~ 7.3)

2.2.1.2 PMSF (100 mM)

2.2.1.3 Distilled Water

2.2.1.4 Sds-PAGE Separation Gel

2.2.1.5 5% Spacer Gel

2.2.1.6 Running Buffer

2.2.1.7 Transfer Buffer

2.2.1.8 Fuchsin Dye

2.2.2 Equipment

2.2.2.1 Cell Scraper

2.2.2.2 Pipettor

2.2.2.3 Microcentrifuge Tube

2.2.2.4 Gel Electrophoresis Fixed Equipment

2.2.2.5 Beaker

2.2.2.6 Electrophoresis Tank

2.2.2.7 Cut The Filter Paper According To the Experiment Requirement

2.2.2.8 Cellulose Nitrate Film Cut According To the Experimental Requirements

2.2.2.9 Clip for Transfer, Two Spongy Pads, a Glass Rod, Filter Paper and Soaked Film

2.2.2.10 Centrifuge

2.3 Design of Experimental Scheme

2.3.1 Extraction of Total Protein from Monolayer Adherent Cells

2.3.1.1 The sample cells are removed from the cell culture chamber, the culture solution is poured out, and the bottle is inverted on the absorbent paper to drain the culture solution.
2.3.1.2 3 mL pre-cooled PBS (0.01M pH 7.2 ~ 7.3) was added into each bottle. Placed the cell culture bottle flat on the table and gently shook it for dozens of seconds to wash the cells and then poured away the lotion. Repeated this procedure twice to wash the culture solution thoroughly. After PBS was emptied, the culture bottle was placed on ice.[7-8]

2.3.1.3 Mixed the cracking liquid and PMSF at a ratio of 1ml: 10 ul. Mixed the cracking liquid and place it on the ice.

2.3.1.4 Each bottle of cells was added with 500 ul PMSF lysate and placed on ice for 30 min for lysis. The culture bottle was shaken back and forth in the process of lysis to ensure adequate cell lysis. The cells were broken up with a Cell Ultrasound to release proteins from the cytoplasm.

2.3.1.5 After lysis, the cells were scraped to the side of the culture bottle with a squeegee-cleaned rod with distilled water, and then the lysates and cell fragments were transferred to a 1.5mL centrifuge tube with a pipette.

2.3.1.6 The lysate and cell fragments in the centrifuge tube were put into a centrifuge and centrifuged at 12000 RPM at 4℃ for 15 min. After centrifugation, the supernatant was transferred into a 1.5mL centrifuge tube and stored at -20℃.

2.3.2 Prepare the protein sample

2.3.2.1 Preparation
1. Clasped the glass with one hand and dip the other hand in dishwashing liquid to gently and slowly scrub. Washed both sides with detergent and rinsed them with running water. Rinsed them with distilled water and dry them on a shelf.

2. Aligned the bottom edges of both the front and rear glass plates and put them into the clamp for clamping. Then stuck the glass vertically on the shelf. The distilled water was filled with a pipette and left stationary for a period of time. Observe that if there was no water leakage then the glass plate would tighten

3. Washed the small beaker used for glue mixing and baked it in the Drying Oven for about five minutes to make sure there was no water in the beaker and prevented the glue from condensing due to dilution. Prepared for glue mixing[9].

2.3.2.2 Glue Configuration
1. SDS-PAGE Separation Glue was first prepared, because the concentration of Separation Glue was relatively large, and the pore size of glue was relatively small. When voltage was applied, small proteins could pass through the hole to the bottom, while proteins with large molecular weight ran slowly. In this way, proteins were separated by differences in molecular weight.

2. Added 10% Separation Glue according to the instruction sheet, shook well immediately after adding TEMED, and started filling with 1 ml pipette. Absorbed 5 ml glue to release along the glass for multiple times, and stoped when the horizontal liquid level of the glue separator reached the middle of the green belt. Then a layer of distilled water was added to the glue.

3. When a refracted ray was observed between the water and the gelatin, the gelatin was said to have solidified. The distilled water could be removed with a pipette when the glue was fully solidified for another 3 minutes after the refraction rayed appear.

4. Prepared 5% concentrated glue according to the specification table, because the concentration of concentrated glue was relatively low and the aperture was relatively large, ensured that all proteins could be on the same level before entering the separation glue, added TEMED and shook well immediately, and started filling glue with 1 ml pipet-gun.

5. After filling the remaining space with the concentrated adhesive, inserted the comb into the
concentrated adhesive. It should be noted that the concentrated adhesive should flow down the glass plate to avoid the formation of bubbles in the adhesive, which would affect the strip results. When inserting the comb, be careful to keep the comb inserted horizontally.

6. After the concentrated glue solidifies, held both sides of the comb in both hands and pulled it out vertically and gently.

7. The gel holder containing the concentrated and separated glue was placed into the electrophoresis tank. Opened the electrophoresis tank to start the gel electrophoresis.

8. After measuring the protein content, the volume of solution containing 10 g protein was calculated as the sample volume. The sample was taken out into a 1.5mL centrifuge tube and 5×SDS sample loading buffer was added until the final concentration was 1×. Before loading, the sample was heated at 99°C in a metal bath for 5-10 minutes to denature the protein.

2.3.2.3 Transmembrane

1. Prepared two thick filter papers and a PVDF film to match the size of the glue. The cut PVDF membrane was activated by methanol, surface tension was eliminated in distilled water, and then soaked in the transfer liquid.

2. Poured transfer liquid into the enamel pan and placed the transfer clip, two sponge pads, a glass rod, filter paper and soaked film.

3. Opened the clamp so that the black side was level. Put a sponge on it and rolled it back and forth several times to remove the bubbles. Rolled the pad with one hand and held it down with the other. Put three layers of filter paper on the cushion, fixed the filter paper with one hand and rolled out the bubbles with the glass rod with the other.

4. Pried off glass plate first then peeled glue, remember to be gentle when pried the glue, want to be in two side gently repeatedly pry. After a while the glass starts to come loose until the glass comes out. After removing the small glass plate, scrape the concentrated glue gently to avoid scraping the separation glue. Carefully peeled off the adhesive cover on the filter paper, align it with the filter paper by hand, and gently roll out the bubbles with the glass rod. Covered the film over the glue, covered the whole glue and do not move and remove bubbles. Cover the film with 3 sheets of filter paper and remove bubbles. Finally, covered another sponge pad, rolled a few times to close the clip. The entire operation in the transfer fluid needed to continue to roll out the bubble. The transfer fluid contained methanol, gloves were worn when operating, and the laboratory was opened for ventilation.

5. Placed the clip into the transfer slot so that the black side of the clip faced the black side of the slot and the white side of the clip faced the red side of the slot. Heat was generated during electrical transfer, and a piece of ice was placed on one side of the tank to cool it. Usually 400 mA was used to transfer 1 h.

6. After the transfer, the membrane was dyed with 1× Fuchsin Dye solution for 5 min on the decolorizing shaker to dye the protein on the membrane. If the color was too dark to see the strip clearly, the membrane could be washed repeatedly for many times until the strip with different color depth caused by different sample amount was seen.

7. Optimization of protein extraction Effects of NaOH concentration, ratio of liquid to material, extraction temperature and extraction time on protein extraction rate of larvae of black water gadfly were studied. Based on the results of the single factor test, the four factors were used as the investigation variables, and the four factors and three levels Box-Behnken response surface test design scheme were used to optimize the protein extraction process. The horizontal design of test factors is shown in Table 1.

| Factors Codes | Levels |
|---------------|--------|
| X1: NaOH mass concentration (g/100mL) A | 2.00 2.25 2.50 |
| Liquid-to-solid ratio(mL/g) B | 15 20 25 |
| X3: Extraction temperature (℃) C | 40 50 60 |
### 3. Attention

1. When extracting the total protein of the monolayer adherent cells, PMSF should be shaken until no crystallization before mixing with the lysate.

2. To extract the total protein from the single-layer adherent cells, scrape the cells on the side of the flask with a clean scraper, move quickly and do your best to keep the process on the ice.

3. The centrifuge should be turned on and pre-cooled.

4. Align the two glasses when inserting into the glass plate in order not to leak glue.

5. The glue filling can be faster at the beginning, the glue surface to the required height to slow down. When operating, the glue must flow down the glass plate, so that there will be no bubbles in the glue. Add water to seal slowly, otherwise the glue will be washed.

6. When inserting a comb, it depends on the type of comb and the number of comb holes.

7. The beaker should be dried after cleaning before gluing to ensure that there is no water residue leading to the decrease of solution concentration.

8. Cells should not be washed for more than a minute during the protein extraction process, which can cause them to wash away.

9. In order to fully lyse cells, ultrasonic cell fragmentation apparatus can be used for ultrasonic destruction of cells.

10. The concentrated adhesive is placed in the electrophoresis tank with the small glass plate facing inward and the large glass plate facing outward. If only one piece of glue is running, the other side of the slot shall be symmetrically installed with a plastic board and the letted side facing outwards.

11. If the sample is added too fast, the sample will rush out of the hole. If there are bubbles, the sample may overflow. When adding the next sample, the sampler should be washed in the external tank electrophoresis buffer for 3 times to avoid cross contamination.

12. Always wear gloves when cutting filter paper and film, as the egg whites on your hands can contaminate the film.

13. The number and size of the PVDF membrane depends on how many target proteins to run and how many lanes to run.

14. After activating the cut PVDF membrane in methanol, eliminating the surface tension in distilled water, and then soaking it in the transfer solution, the cut PVDF membrane is held by tweezers and placed gently in a dish with ultra-pure water to make the membrane float on the water and only the lower layer comes into contact with the water. This wets the entire membrane by capillary action. If the film sinks into the water, an air film forms between it and the water, which prevents it from absorbing water.

15. Must be careful when peeling off the plastic and prying the glass plate, which is easy to crack.

### 4. Conclusion

The results were as follows: one group of cells expressed proteins, and the other group did not. The expression of specific protein in animal body will be affected by external factors. Also, Western Blot can be a way to show this change.
Fig. 1 Effects of extraction method (A), NaOH mass concentration (B), liquid-to-solid ratio (C), extraction temperature (D) and extraction time (E) on the extraction rate of proteins from Hermetia illucens larvae.

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