

1. Introduction

Streptococcus suis 2 is an important emerging zoonotic pathogen [1]. It mainly causes meningitis in pigs [2]. The process of meningitis caused by SS2 involves multiple steps. The interaction between SS2 and Brain microvascular endothelial cells (BMECS) is the essential step to disrupt the BBB and enter the central nervous system (CNS) [3]. Blood-Brain Barrier (BBB) is a complex and highly organized multicellular structure that shields CNS from harmful substances and invading organisms from the bloodstream [4]. Brain microvascular endothelial cells are the most important component of BBB. Therefore, the study of the interaction between SS2 and brain microvascular endothelial cells is of great significance to reveal the mechanism of meningitis [5].

The extraction of total RNA is one of commonly used techniques in molecular biology experiment [6]. RNA is prerequisite to facilitate any study related to gene expression and also for downstream applications such as RT-PCR, library construction, real-time PCR and northern hybridization [7]. Therefore, undegraded and intact RNA free of DNA, protein and other impurities are particularly important.

To study the pathogenesis of meningitis caused by SS2, we use SS2 to infect bEnd.3 to get stable cDNA for next research on differences in gene expression and protein expression of cytokines.

The aim of this study was to extract the total RNA from mouse brain-derived Endothelial cells (bEnd.3) infected by *Streptococcus suis* serotype 2 (SS2) and transcript to complementary DNA (cDNA).

2. Methods

2.1. Cultivation of bEnd.3 and inoculation of streptococcus

Mouse brain-derived Endothelial cells (bEnd.3) were thawed and cultured in dulbecco's modified eagle medium (DMEM) containing 8 % serum in carbon dioxide incubator. When the cells are in the logarithmic growth phase and occupy 80 % of the bottom of the bottle, the cells are digested with trypsin and spreaded into a 6-well plate with 2ml cell suspension per well. SS2 was activated and the concentration of the bacterial solution was measured. Bacterial solution were inoculated into the 6-well plate at a multiplicity of infection (MOI) of 100. After 12 hours, cells were harvested and total RNA were extracted.

EXTRACTION AND REVERSE TRANSCRIPTION OF TOTAL RNA FROM MOUSE BRAIN-DERIVED ENDOTHELIAL CELLS.3 INFECTED BY *STREPTOCOCCUS SUIS* 2

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Abstract: *Streptococcus suis* 2 is an important emerging zoonotic pathogen. It mainly causes meningitis in pigs. We use SS2 to infect bEnd.3 to get stable cDNA for next research on differences in gene expression and protein expression of cytokines.

The paper presents an SS2 study for bEnd.3 infection to obtain stable cDNA for subsequent study of differences in gene expression and cytokine protein expression.

Objective: The aim of this study was to extract the total RNA from mouse brain-derived Endothelial cells (bEnd.3) infected by *Streptococcus suis* serotype 2 (SS2) and transcript to complementary DNA (cDNA).

Materials and methods: SS2 strain were obtained from Jilin University, China. BEnd.3 was from Henan institute of Science of Technology, China. Reverse transcription kit was from Takara company, Japan. Trizol was from Bioteke company, China. Nanodrop instrument was from Thermo company, USA. Polymerase chain reaction (PCR) instrument was from Biometra company, Germany.

We used SS2 to infect bEnd.3 at a multiplicity of infection (MOI) of 100 for 12h. Cells were harvested and Trizol method was chose to extract the total RNA of bEnd.3 infected by SS2. Nanodrop instrument was used to measure the concentration of RNA and the values of OD260/280 and OD260/230. RNA were transcribed to cDNA with reverse transcription kit by PCR instrument.

Results: trizol method used in this study was reliable and high-quality RNA were obtained. Stable cDNA were obtained by reverse transcription kit.

Conclusion: in this experiment high-quality RNA was obtained and reverse transcribed to stable cDNA for subsequent detection of related cytokines. This study provides an approximate RNA extraction method and good experimental foundation for downstream research.

Keywords: extraction, reverse transcription, RNA, brain-derived endothelial cells, *Streptococcus suis* 2.

2.2. Extraction of total RNA

The TaKaRa MiniBEST Universal RNA Extraction kit and Trizol RNA extraction methods were compared. The RNA obtained by the Trizol method was of higher quality, so the Trizol method was selected to extract total RNA from cells.

2.3. Reverse Transcription (RT)

Takara's PrimeScript RT Master Mix reverse transcription kit was used for reverse transcription. The reaction system of RT is listed in Table 1.

Table 1
Reaction System of RT

Reagent	Content(ul)
Reverse Transcriptase (5x)	4
Total RNA	★
RNase Free dH2O	Up to 20

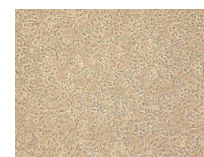
Note: ★ – 1000/concentration of RNA

The reverse transcription was carried out by PCR instrument with the conditions as follows: 37 degree with 15 minutes, 85 degree with 5 seconds and 4 degree with reaction stop.

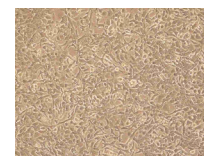
3. Results

3.1. Culture of bEnd.3

Thawed bEnd.3 grew very well. Cells have obvious shrinkage compared with control at 12 hours after SS2 infection (Fig. 1).



a



b

Fig. 1. bEnd.3 at 12 hours after SS2 infection: a – cell with SS2 infection; b – control

3.2 Concentration and purity of RNA

Extracted total RNA were detected by Nanodrop instrument and the concentration and purity were obtained (Tables 2, 3).

Table 2
Concentration of RNA

Sample	Repeats	Average(ng/ul)
1	3	1183
2	3	1023.9
3	3	1213.3
4	3	1314.2
5	3	931.1
6	3	1273.5

Table 3
Purity of RNA

Sample	OD260/280	OD260/230
1	1.82	2.18
2	1.95	2.09
3	1.86	2.12
4	1.87	2.17
5	1.93	2.18
6	1.89	2.10

4. Discussion

RNA is very instable and easily degraded by RNase [8]. It is difficult to obtain high quality RNA. Therefore, during the operation, lots of attentions must be cared. The operation area should be clean enough. Pipette should be dedicated. Tips, tubes, gloves and masks must be disposable and masks and gloves should be replaced frequently. Tubes must be picked up by tweezer instead of hand.

To obtain high-quality RNA, all kinds of contaminations must be avoided. The value of OD260/280 for pure RNA is 2.0. If the ratio is low, it means that it is contaminated by protein (aromatic) or phenolic substances [9]. The value of OD260/230 for pure RNA is 2.5. If the ratio is less than 2.0, it indicates that the sample is contaminated by carbohydrates and salts. Though the extraction of RNA followed regulation strictly, a certain degree of protein and carbohydrate contamination still cannot be avoided.

The extracted total RNA is very unstable, it is best to perform reverse transcription immediately and store it at low temperature.

Study limitations. There may be possible limitation in this study. Although the values of OD260/280 and OD260/230 are quite high, there is still lack of visible evidence of RNA integrity. Gel electrophoresis should be performed to complete the result

of RNA integrity. If 3 bands for 5S rRNA, 18S rRNA and 28S rRNA can be seen, it means there is no degradation of extracted RNA. At the same time, the brightness ratio of the two bands of 18S rRNA and 28S rRNA can be used to determine the integrity of RNA [10].

Prospects for further research. RNA extraction methods mainly include Trizol method and extraction kit. Trizol method takes nearly 3 hours to extract RNA. Also the risk of pollution is very high because reagents used during the operation are self-prepared. The advantages of the kit are short time-consuming, less pollution, and convenient operation, but the disadvantages are also obvious: too few consumables and high cost. Hopefully upgraded products can be developed quickly and make the RNA extraction to be cheap, convenient and efficient.

5. Conclusions

In this experiment, *Streptococcus suis* were inoculated to bEnd.3 at a MOI of 100 for 12 hours, and then RNA was extracted from cells infected with *Streptococcus suis* 2 using Trizol method. High-quality RNA was obtained and reverse transcribed to stable cDNA for subsequent detection of related cytokines. This study provides an appropriate RNA extraction method and good experimental foundation for downstream work.

References

- Liu, F., Li, J., Yan, K., Li, H., Sun, C., Zhang, S. et. al. (2017). Binding of Fibronectin to SsPepO Facilitates the Development of *Streptococcus suis* Meningitis. *The Journal of Infectious Diseases*, 217 (6), 973–982. doi: <http://doi.org/10.1093/infdis/jix523>
- Dutkiewicz, J., Zając, V., Sroka, J., Wasiński, B., Cisek, E., Sawczyn, A. et. al. (2018). *Streptococcus suis*: a re-emerging pathogen associated with occupational exposure to pigs or pork products. Part II – Pathogenesis. *Annals of Agricultural and Environmental Medicine*, 25 (1), 186–203. doi: <http://doi.org/10.26444/aaem/85651>
- Coureuil, M., Lécuyer, H., Bourdoulous, S., Nassif, X. (2017). A journey into the brain: insight into how bacterial pathogens cross blood–brain barriers. *Nature Reviews Microbiology*, 15 (3), 149–159. doi: <http://doi.org/10.1038/nrmicro.2016.178>
- Sarkar, A., Fatima, I., Mohammad Sajid Jamal, Q., Sayeed, U., Kalim A. Khan, M., Akhtar, S. et. al. (2017). Nanoparticles as a Carrier System for Drug Delivery Across Blood Brain Barrier. *Current Drug Metabolism*, 18 (2), 129–137. doi: <http://doi.org/10.2174/1389200218666170113125132>
- Sui, Y., Zhang, Z., Wang, H. et. al. (2017). Effects of Suliyisin on Apoptosis of Human Cerebral Microvascular Endothelial Cells. *Letters in Biotechnology*, 3 (28), 290–294.

6. Lin, Z., Zhang, H., Jie, Y. et al. (2020). Modified Trizol Method for Extracting RNA from Mouse Skin. *Journal of SUN YAT-SEN University*, 41 (2), 224–232.
7. George, A. (2018). Simple and efficient method for functional RNA extraction from tropical medicinal plants rich in secondary metabolites. *Tropical Plant Research*, 5 (1), 8–13. doi: <http://doi.org/10.22271/tpr.2018.v5.i1.002>
8. Liang, Y., Huang, L., Liu, T. (2021). Corrigendum: Development and Delivery Systems of mRNA Vaccines. *Frontiers in Bioengineering and Biotechnology*, 9, 718–728. doi: <http://doi.org/10.3389/fbioe.2021.766764>
9. Fang, T., Xu, Q., Jin, A. et. al. (2021). Comparison of total RNA extraction quality from different tissues of moso bamboo. *Journal of Central South University of Forestry & Technology*, 9 (41), 157–165.
10. Guo, L., Bai, F., Sha, X. et. al. (2021). Comparison of RNA extraction methods from tomato leaves and fruits. *Hubei Agricultural Sciences*, 60 (3), 141–144.

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