Advanced Materials Research Vol. 825 (2013) pp 182-185

doi:10.4028/www.scientific.net/AMR.825.182

Optimization of the Separation Method for Bioleaching Bacteria DNA and RNA Extracted Simultaneously

Hui Yun[1,2,a*] ,Jianping Xie[1,2,b*], Ning Huang[1,2,c], Xinjie Yang[1], Jing Xu[1,2], Guanzhou Qiu[1,2,d], Xinxing Liu[1,2,e]

[1]School of mineral processing and bioengineering, Central South University, Changsha, Hunan, 410083, China

[2]Key Laboratory of Biometallurgy, Ministry of Education, Changsha, Hunan 410083, China

[a]csuyunhui2011@gmail.com,[b]Whitewolf1101@foxmail.com, [c]nhuangy@gmail.com, [d]qgz@csu.edu.cn, [e]x-mine@mail.csu.edu.cn

*These two authors contribute equally to this study.

Keywords：biohydrometually, DNA, RNA

Abstract. DNA and RNA based analysis was a useful way to characterize microbial communities during biohydrometallurgical processes. However, feasible, affordable and efficient DNA and RNA separation methods are rarely reported although several simultaneous DNA and RNA extraction methods have been developed recently. In this study, various salts including NaCl, $CaCl_2$ and LiCl were tested for separation of DNA and RNA. Salt concentration, nucleic acid concentration, precipitation temperature and time were optimized. The results showed that LiCl was more efficient to separate DNA and RNA than the other two salts. The optimized conditions were as follows: 1/4 volume saturated LiCl was used for precipitating RNA first at -20℃ for 30 min for total nucleic acid concentration of approximately 200-400 ng/μL, and then centrifuged at 12,000×g at room temperature for 20 min to collect RNA. DNA in the supernatant was precipitated using 0.6 volume isopropanol and then collected by centrifugation at 12,000×g at room temperature for 20 min. The results indicated that DNA and RNA could be extracted from not only pure culture of *Acidithiobacillus ferrooxidans (At. ferrooxidans), Acidithiobacillus caldus (At. caldus)*, *Acidithiobacillus albertensis (At. albertensis), Leptospirillum ferrooxidans (L. ferrooxidans), Ferroplasma thermophilum (F. thermophilum)*, but also the acid mine drainage(AMD) water samples from Daye copper mine, Xiangxi gold mine and Axi gold mine. The resulted DNA and RNA could be amplified and $A_{260/280}$ was 1.8-2.0 and $A_{260/230}$ was 1.8-2.2, which indicated high quality of DNA and RNA. This method could be widely used for separation of bioleaching bacteria DNA and RNA extracted simultaneously.

1. Introduction

The intact and integrate nucleic acid information is vital and influential when biological molecular techniques were used in researching environmental samples such as the community structure analysis, the dynamic changes of the communities and functional genes, etc. Any biased data may result in the meaningless of the investigation. The extraction and separation of nucleic acid were the basis for such researches, which should be reliable and reproducible. Though numerous methods for nucleic acid recovery have been put forward [1], the methods for simultaneously recovery and separation of DNA and RNA were rarely reported [2,3]. This paper mainly introduced a method for separation of DNA and RNA for the nucleic acid simultaneously recovered.

2. Material and methods

Sample origin. The pure culture of *Acidithiobacillus ferrooxidans (At.ferrooxidans), Acidithiobacillus caldus (At. caldus)*, *Acidithiobacillus albertensis (At. albertensis), Leptospirillum ferrooxidans (L. ferrooxidans), Ferroplasma thermophilum (F. thermophilum)* were obtained from the Ministry of Education Key Laboratory of Biometallurgy strain library, Central South University. The environmental samples were taken from Daye copper mine, Hubei Province, Xiangxi gold mine, Hunan Province and Axi gold mine, Xinjiang Uygur Autonomous Region.

Optimization of separation. The nucleic acid of *At. ferrooxidans* was used to optimize the procedure such as different salts, different primary concentration of nucleic acid, different concentration of LiCl, low temperature incubation time and other controllable factors that could influence the separation of RNA from DNA. I) The concentration of NaCl solution changed from 1M to 5M in 1M increments as well as saturated solution and the volume was 1/3 that of nucleic acid. The incubation time was 1 h and RNA was collected by centrifugation at 12,000×g for 20 min at room temperature. DNA in the supernatant was precipitated with isopropyl alcohol. Both DNA and RNA were purified with 70% ethanol. II) The tested concentrations of $CaCl_2$ solution were 0.5M, 1M, 1.5M, 2M, 4M, 6M, 8M and 10M with equal volume added. After room temperature incubation for 30 min, RNA and DNA were recovered as mentioned previously. III) The concentrations of 10M,12.5M,15M,17.5M and saturated LiCl solution were compared with 1/4 volume added. The mixture was first incubated at -20℃ and then 4℃ with time interval of 0, 2, 4, 6, 8, 10 and 12 h. RNA and DNA were recovered in the same way as mentioned above.

Separation and purification of RNA from DNA with Kit. Total crude nucleic acid extracted was separated using QIAGEN All Prep DNA/RNA Mini Kit (Germany) according to the manufacturer's protocol. The eluted RNA and DNA were precipitated with 1/10 volume 3M sodium acetate and 2.5 volume absolute ethanol.

Assessment of DNA and RNA. Nucleic acid was analyzed by electrophoresis with the molecular weight ladders and Nanodrop 1000 spectrometer. The ratios of $A_{260/280}$ and $A_{260/230}$ ranged 1.8-2.0 and 1.8-2.2, respectively, which was considered reasonable for the downstream molecular analysis.

PCR amplification and reverse transcription. RNA was reverse transcribed using primer *gln* A-F (5'-CATCCGCATTCCCTTCGTCAAC-3') for the glutamine synthetase gene with RT reagent Kit (Takara)1 μL aliquot of the reverse transcription products from a total volume of 20 μL was used for PCR amplification with the forward primer *gln* A-F and the reverse primer *gln* A-R (5'-GGCAGGTCGTAAAGATTCTTGTCC-3'). The quality of DNA was also examined by PCR amplification of 16SrRNA gene, using bacterial universal primers 27F and 1492R： 5' -AGAGTTTGATCMTGGCTCAG-3', 5' -GGTTACCTTGTTACGACTT-3'.

3. Results

Optimization of separation conditions. The purity and intactness were considered important factors when choosing separation conditions. Only part of RNA could be precipitated when using NaCl [4] and most still coexisted with DNA. Though $CaCl_2$ [5] could efficiently function on RNA, some DNA remain with RNA, thus caused loss of DNA. In LiCl separation experiments, the effects of different nucleic acid concentration on separation were compared. Based on the operational volume of 100 μL， at concentration lower than 400 ng/μL it was easy to lose the unseen pellet. However, the higher the concentration of nucleic acid, the more viscous the solution, and RNA and DNA intertwined for their analogous structure and could be precipitated together. According to this, the accepted concentration of nucleic acid was some 400 ng/μL. Different concentrations of LiCl were used to treat the crude extracts. Concentrations lower than saturation level (approximately 20M) tended to precipitate RNA insufficiently and influenced the separation in different degrees. As for the incubation time at 4℃, after incubation at -20℃ for 30 min, RNA could not be precipitated effectively without the 4℃ incubation. 4℃ [6] was postulated a pivotal point for nucleic acid dissolution and sedimentation, which could be proved by the phenomenon that incubation time longer than 8 h led to the redissolution of nucleic acid. As the results shown, after -20℃ incubation, 4℃ was necessary for the precipitation. From the foregoing, the optimized separation conditions were using saturated LiCl and incubation at 4℃ for about 8 h.

Separation and purification. Compared with LiCl separation, the main advantages of Separation Kit lied in the time-saving and high efficacy when dealt with small amount, but was costly and limited in handling capacity. Though a little time-consuming, the amount of initial nucleic acid was flexible and DNA and RNA could be separated more efficiently using LiCl.

Assessment of DNA and RNA. As the electrophoresis results shown, DNA exhibited intact band (≥23kb) and RNA exhibited 23S and 16S bands and both had no obvious shearing. RNA and DNA separated with kit had low $A_{260/280}$ and $A_{260/230}$, which could be optimized by desalting. While using LiCl, the $A_{260/280}$ was approximately 1.8 to 2.0 and the $A_{260/230}$ was approximately 1.8 to 2.2.

PCR amplification and reverse transcription. Both DNA and RNA separated with LiCl were of high quality and suitable for the downstream analysis such as PCR, reverse transcription-PCR and Restrict Fragment Length Polymorphism. We obtained the amplification products of 16S rRNA gene (about 1500 bp) and 174 bp RT-PCR products. (Fig.1, 2)

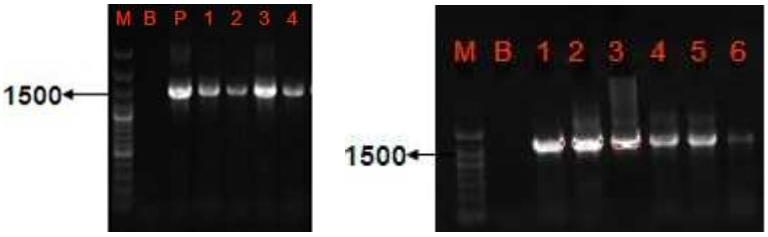

Fig.1 Left) The PCR amplification of separated DNA using LiCl for pure cultures *At. ferrooxidans, At. caldus*, *At. albertensis, F. thermophilum and L. ferrooxidans* ; Right) the PCR amplification of separated DNA using LiCl of acidic environmental samples

Note:B means blank control, P means positive control;

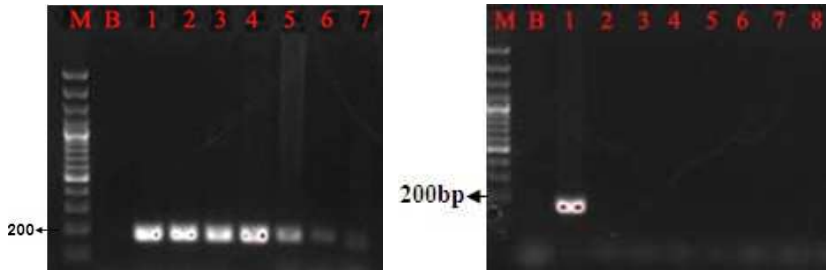

Fig.2 Left) The reverse transcription PCR products of *At. ferrooxidans, At. caldus* and *At. albertensis* and four environmental samples for glutamine synthetase gene; Right) PCR amplification directly from RNA without reverse transcription;

Note:B means blank control,1 in the right graph means positive control;

4. Discussion

The key mechanism to RNA and DNA separation using chloride salts lied in that cation could competitively bind with nucleic acid bound water or neutralize the negative charge of nucleic acid, thus changed the configuration of nucleic acid, weakened the repulsive interaction between nucleic acid molecules and contributed to precipitation [7]. The main difference between Na^+, Ca^{2+} and Li^+ was the ion size and the charges. It is likely that Li^+ had a suitable size and sufficient charge to function on RNA, though it may also bind with DNA within the minor groove and change DNA in a different configuration but not globalization [8]. The results showed that the saturated solution had better consequence which was consistent with what Cathala et al [9] reported. The viscosity of nucleic acid solution had great impact on separation, because RNA and DNA or RNA and RNA could form double strand which brought in co-precipitation in high concentration. When it comes to the incubation time at 4℃, followed -20℃ incubation for 30 min, 4℃ was propitious to form crystal nucleus. Longer time did not mean the increase of precipitation, but at least 8 h was needed, which, references reported as overnight [10]. The quality and recovery rates of RNA and DNA separated with LiCl surpassed with kit, which were eligible for downstream analysis.

5. Conclusion

The developed DNA and RNA separation method by using LiCl is an efficient and affordable way to replace the QIAGEN All Prep DNA/RNA kit. LiCl was more efficient to separate RNA from DNA than NaCl and $CaCl_2$. The optimized conditions were as follows: 1/4 volume saturated LiCl was used for precipitating RNA first at -20℃ for 30 min and then 4℃ for 8 h for total nucleic acid concentration of approximately 200-400 ng/μL, and then centrifuged at 12,000×g at room temperature for 20 min to collect RNA. DNA in the supernatant was precipitated using 0.6 volume isopropanol and then centrifuged in the same way to collect DNA. The results indicated that DNA and RNA of pure culture or AMD samples could be efficiently separated and amplified. This method could be widely used for separation of bioleaching bacteria DNA and RNA extracted simultaneously.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 51104189), the 53rd China Postdoctoral Science Foundation (No. 2013M531814), and the National Basic Research Program of China (No. 2010CB630901).

Main References

[1] Richard A.Hurt, Xiaoyun Qiu, Liyou Wu, Yul Roh,A.V.Palumbo and Jizhong Zhou. 2001. Simultaneous Recovery of RNA and DNA from Soils and Sediments. Applied and Environmental Microbiology 87(10):4495-4503.

[2] VT Tictor,G Atanasova,A Aqil,V Maquet. 2009. Development of a procedure to simultaneously isolate RNA, DNA, and proteins from characterizing cells invading or cultured on chitosan scaffolds.Anal.Biochem 395(2):145-147.

[3] CM. Zammita, LA. Mutcha, HR Watling,ELJ Watkin. 2011. The recovery of nucleic acid from biomining and acid mine drainage microorganisms.Hydrometallurgy 108(1-2):87-92.

[4] Robert I.Griffiths, Andrew S.Whiteley, Anthony G.Donnell. 2000. Rapid Method for Co-extraction of DNA and RNA from Natural Environments for Analysis of Ribosomal DNA- and rRNA-Based Microbial Community Composition. Applied and Environmental Microbiology 66(12):5488-5491.

[5] V Dal Cin,M Danesin,FM Rizzini,A Ramina.2005. RNA extraction from plant tissues,the use of calcium to precipitate contaminating pectic sugars.Molecular biotechnology 31:113-119.

[6] A Eon Duval,K Gumbs,C Ellett. 2003. Precipitation of RNA impurities with High salt in a plasmid DNA purification process: using of experimental design to determine reaction conditions .Biotechnology and Bioengineering 83(5): 544-553.

[7] Chang,Puryear, Cairney. 1993. A Simple and Efficient Method for Isolating RNA from Pine Trees.Plant Molecular Biology Reporter 11(2):113-116.

[8] YK Hong,SD Kim,M Polne-Fuller,A Gibor. 1995. DNA extraction conditions from Porphyra perforate using LiCl.Journal of Applied Phycology 7:101-107.

[9] G Cathala,JF Savouret,B Mendez,BL West. 1983. A method for isolation of intact,translationlly active ribonucleic acid.Laboratory methods 2(4):329-335.

[10] Chang,Puryear,Cairney. 1993. A simple and efficient method for isolating RNA from pine trees.Plant molecular biology reporter 11(2):113-116.