Assessment Of Total RNA Extraction From Edible Mushroom (*Agaricus bisporus*) With Three Current Methods

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ABSTRACT: Nucleic acid extraction is an attractive alternative to labor-intensive manual methods. In this report, a few methods of total RNA Extraction from Edible mushroom (*Agaricus bisporus*) compared. This methods consist AGPC(Acid Guanidinium-Phenol-Chloroform), The method according by Lithium chloride and RNx total RNA isolation Kit (CinnaGen). Best method selected for the preparation of high quality and intact total RNA from *A. bisporus*. Based on the Ethidium Bromide straining pattern of 28s and 18s rRNAs, either denaturing (formaldehyde/agarose) or nondenaturing (TAE/agarose) gel electrophoresis could be used to determine the general integrity of the RNA preparation. Analysis by either method revealed sharp rRNA bands indicative of high quality RNA. Yield of RNA was acceptable in any three method but RNA quality in AGPC method similar with RNx Total RNA isolation kit (CinnaGen).

Keywords: white button mushroom, RNA extraction, Guanidinium-Phenol-Chloroform(AGPC), RNx

INTRODUCTION

*Agaricus bisporus* is cultivated around the world, and its share of the international mushroom market is 31.8% (Rai, 2004). Like all fungi, *A. bisporus* is a heterotrophic organism; it lacks chlorophyll and thus cannot use photosynthesis to convert carbon dioxide to organic carbon (Bechara, 2006). Efficient methods for the genetic transformation of filamentous fungi have been developed in the last 15 years. This had prompted a burst of molecular investigations with these organisms for the purpose of fundamental experimentation, as well as for applied research. In many cases, experiments require the study of minute amounts of nucleic acid from a large number of samples (screening of particular events of transformation, detection of different mutant alleles, analysis of transcripational regulation of messenger RNA, etc.) (Lecellier, 1994) Several molecular techniques used to isolate high-quality RNA, free from DNA from *A. bisporus*. In this report, I compared three different methods to isolate RNA from common button mushroom, including to isolation using: 1. The Acid Guanidinium-Phenol-Chloroform(AGPC), 2. Method described previously by (Sreenivasaprasad, 2000) according to Lithium Chloride (Licl) and 3. RNx Total RNA isolation Kit (CinnaGen).

Indeed, The aim of this study was to compare the performance of RNA extraction by three methods to isolate Total RNA from *A. bisporus*. 
MATERIALS AND METHODS

**Fungal strain and culture condition**

The *A. bisporus* Horst U1 strain IM008 that obtained from the research group of industrial fungi biotechnology, ACECR (project number 822-55), was used in this study. The media used for mushroom culture were compost extract/complete yeast extract media (CE/CYM).

**RNA extraction**

The methods to study are including:

- **AGPC method:** Guanidinium thiocyanate and chloride are among the most effective protein denaturant. As a strong inhibitor of ribonucleases, guanidinium chloride was first introduced as a deproteinization agent for isolation of RNA by cox. Since then guanidinium extraction has become the method of choice for RNA purification, replacing phenol extraction. Guanidinium methods have been used successfully by chirgwin et al. to isolate undegraded RNA from ribonuclease-rich tissues.

  The denaturing solution (solution D) was 4 M guanidinium thiocyanate, 25 mM sodium citrate, pH 7; 0.5% sarcosyl, 0.1 M 2-mercaptoethanol. Solution D was prepared by adding 0.36 ml 2-mercaptoethanol/50 ml of stock solution. Phenol (nucleic acid grade, Bethesda Research Laboratory) saturated with water was kept at 4°C up to 1 month. The following protocol describes isolation of RNA from 100 mg of mycelium of fungi.

  Immediately after removal mushroom liquid culture, the Edible mushroom mycelium was homogenized and placed into a 1.5 polypropylene tube. Therefore 1 ml of solution D added to micro tube. Sequentially, 25 μl of 2 M sodium acetate, pH 4, 250 μl of phenol (water saturated), and 250 μl of chloroform-isooamyl alcohol mixture (49:1) were added to the homogenate, with thorough mixing by inversion after the addition of each reagent. The final suspension was shaken vigorously for 10 s and cooled on ice for 15 min. Samples were centrifuged at 10,000 g for 20 min at 4°C. The aqueous phase was transferred to a fresh tube, mixed with 1 ml of isopropanol, and then placed at -20°C for at least 1 h to precipitate RNA. Sedimentation at 10,000 g for 20 min was again performed and the resulting RNA pellet was dissolved in 300 μl of solution D, and precipitated with 1 vol of isopropanol at -20°C for 1 h. After centrifugation in an Eppendorf centrifuge for 10 min at 4°C the RNA pellet was washed with 75% ethanol. After RNA pellet dried dissolved in 40-50 μl DEPC water (RNase free).

  The Second method that used in this study according to the method described previously by (Sreenivasaprasad, 2000). Fungi mycelium were ground to a fine powder in a pestle and mortar under liquid N₂. 150 mg of the ground fungal material was transferred to a 1.5 ml centrifuge tube containing: 450 μl of extraction buffer (0.2 M NaAc, 10 mM EDTA, 1% (v/v) SDS and 0.5% (v/v) p-mercaptoethanol) and 450 μl of phenol:chloroform:isoamyl alcohol mixture 25:24:1, pH 5.2 (Sigma) pre-heated to 65°C. The sample was incubated in a waterbath at 65°C for 15 minutes and centrifuged at 15,000 g for 30 minutes at 4°C. The aqueous layer was transferred to a 1.5 ml centrifuge tube and an equal volume of chloroform:isoamyl alcohol 24:1 was added to remove any residual phenol and the mixture was centrifuged at 15,000 g for 30 minutes. The aqueous phase was removed and 12 M Lithium Chloride (LiCl, Sigma) was added to give a final concentration of 2 M LiCl and mixed thoroughly and incubated overnight at 4°C to precipitate the RNA. The RNA was pelleted by centrifugation at 15,000 g for 30 minutes at 4°C and the supernatant was removed and the pellet washed with 150 μl of 3 M NaAc pH 5.2 by centrifugation at 15,000 g for 15 minutes at 4°C. The supernatant was decanted and the RNA pellet was washed with 400 μl of 70% (v/v) EtOH by centrifugation as above. The 70% (v/v) EtOH wash was repeated to remove salts and trace amounts of organic solvents and the pellet was dried in a desiccator. The RNA pellet was re-suspended in 30-40 μl of DEPC treated water.

  The Total RNA Isolation Kit, as the third method based on the manner described by (Takao, 2006), has been used to isolate RNA from *A. bisporus*.

**Quantification of RNA**

RNA quantification was carried out by preparing a 1:60 dilution of the RNA samples using DEPC treated water and placing it in a quartz cuvette and the absorbance was measured at 260nm and 280nm using a GENOVA spectrophotometer (Molloy, 2004).

To check the quality and quantity of the RNA prepared by these methods, 5μl of RNA was electrophoresed on 1% Agarose gel with Formaldehyde and stained by ethidium bromide (Rapley, 2004).
RESULTS AND DISCUSSION

The amount and purity of the extracted RNA and also the presence of DNA contamination compared. As shown in table 1, yield of RNA was higher using the RNX Isolation kit than obtained with other protocols. Purity (estimated by the ratio A260/A280) of the RNA was less optimal in two common protocols. However, AGPC method have a high quality than Licl method.

For the three different protocols, I compared the amount and purity of the extracted RNA and also the presence of DNA contamination. As shown in table 1, yield of RNA was acceptable in any three method but RNA quality in AGPC method similar with RNx Total RNA isolation kit.

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<table>
<thead>
<tr>
<th>Isolation technique</th>
<th>RNA yield (μg/ml)</th>
<th>A260/A280</th>
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<tbody>
<tr>
<td>1 AGPC</td>
<td>22 ± 3</td>
<td>2.0 ± 0.1</td>
</tr>
<tr>
<td>2 According by Licl</td>
<td>17 ± 2</td>
<td>1.4 ± 0.1</td>
</tr>
<tr>
<td>3 RNx Total RNA isolation kit</td>
<td>54 ± 6</td>
<td>1.9 ± 0.1</td>
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</tbody>
</table>

One A260 unit is 40 μg/ml of RNA

*The values represent averages of seven different RNA isolation

REFERENCES


