

# An Improved Total RNA Extraction Method for White Jelly Mushroom *Tremella fuciformis* Rich in Polysaccharides

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**Abstract** An improved method for extracting high quality and quantity RNA from a jelly mushroom and a dimorphic fungus—*Tremella fuciformis* which is especially rich in polysaccharides, is described. RNA was extracted from *T. fuciformis* mycelium M1332 and its parental monokaryotic yeast-like cells Y13 and Y32. The A260/280 and A260/230 ratios were both approximately 2, and the RNA integrity number was larger than 8.9. The yields of RNA were between 108 and 213 µg/g fresh wt. Downstream molecular applications including reverse transcriptional PCR and quantitative real-time PCR were also performed. This protocol is reliable and may be widely applicable for total RNA extraction from other jelly mushrooms or filamentous fungi rich in polysaccharides.

**Keywords** Dimorphism, Jelly mushroom, RNA extraction, *Tremella fuciformis*

*Tremella fuciformis*, or white jelly mushroom, is popular in China and Southeast Asia because of its nutritional values and enticing flavors. *T. fuciformis* is a typical dimorphic fungus, having the ability to change between a unicellular yeast phase and a multicellular filamentous phase [1]. Dimorphism provides a model to investigate the morphogenesis and cellular differentiation in eukaryotes, and it is directly correlated with the invasion of hosts for pathogenetic dimorphic fungi [2]. Previous studies on *T. fuciformis* dimorphism have focused on the environmental factors and cell wall composition differences [1, 3], while no molecular study has been reported. RNA extraction is a prerequisite step for molecular biological studies. *T. fuciformis*

contains rigid cell wall and high level of polysaccharides which co-precipitate with or bind to RNA [4]. Standard RNA isolation kits such as Trizol Reagent (Invitrogen, Carlsbad, CA, USA) and RNAiso plus (Takara, Dalian, China) do not normally work efficiently for *T. fuciformis* which is rich in polysaccharides. Therefore, a simple protocol was developed for RNA extraction of *T. fuciformis*. Abundant and intact RNA was isolated from mycelium and yeast-like cells of *T. fuciformis* which can be applied for subsequent researches on dimorphism.

A dikaryotic strain of *T. fuciformis* M1332 (mycelial form) and two parental monokaryotic strains Y13 and Y32 (yeast form), were obtained from the Culture Collection of State Key Laboratory of Agricultural Microbiology which is the part of China Center for Type Culture Collection (CCTCC). The strains were incubated at 25°C for 7 days to harvest yeast-like cells and 20 days to obtain mycelium. Samples were collected into a pre-chilled mortar and grounded to fine powder in liquid N<sub>2</sub>. The powder (approx. 0.05 g) was subsequently transferred into a centrifuge tube containing 0.7 mL extraction buffer (1.5% (v/v) sodium dodecyl sulfate, 1 M NaCl, 50 mM EDTA, 100 mM Tris-HCl [pH 8.0]) and suspended thoroughly. After mixed with 0.3 volume of saturated NaCl solution, one volume of phenol/chloroform (1 : 1, v/v) were added to each tube and mixed fully. The tubes were centrifuged at 12,000 ×g for 10 min at 4°C. Then the supernatant was collected carefully in a new tube and 1/3 volume of 8 M LiCl were added.

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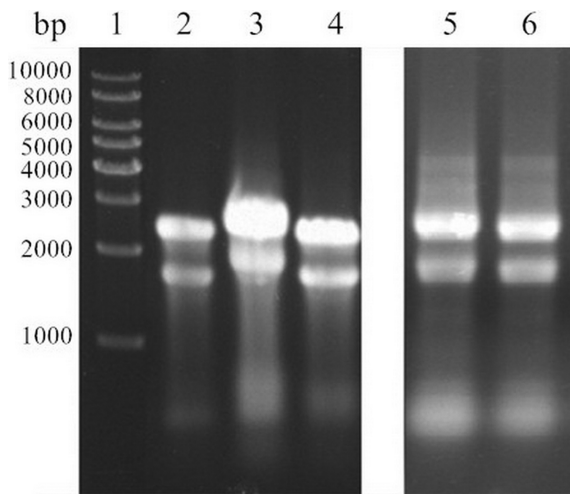
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**Fig. 1.** Electrophoresis of total RNA. Lane 1, DNA marker; lane 2, RNA of *Tremella fuciformis* mecelium M1332 extracted by present protocol; lane 3, RNA of yeast-like cell Y13 extracted by present protocol; lane 4, RNA of yeast-like cell Y32 extracted by present protocol; lane 5, RNA of yeast-like cell Y13 extracted by Trizol reagent; lane 6, RNA of yeast-like cell Y13 extracted by RNAiso plus. The molecular weight of DNA marker (bp) is shown on the left.

After precipitation at  $-20^{\circ}\text{C}$  for 2 hr, the RNA pellet was collected by centrifugation at  $12,000 \times g$  for 10 min at  $4^{\circ}\text{C}$ . The pellet was washed with 1 mL 70% (v/v) ethanol twice, air-dried, and dissolved in 20  $\mu\text{L}$  diethylpyrocarbonate-treated water. The extracted RNA was estimated by 0.8% (w/v) agarose gel. The purity and quantity of RNA was tested by evaluating the ratio of A<sub>260</sub>/280 and A<sub>260</sub>/230 using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). RNA integrity number (RIN) was examined using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA).

This improved method was compared with standard RNA isolation kit including Trizol reagent (Invitrogen) protocol and RNAiso plus (Takara). The protocol described here efficiently eliminated the interference of polysaccharides and produced white and water-soluble RNA precipitates in high yields. The extracted RNA from mycelium and yeast-like cells showed sharp and clear 28S and 18S ribosomal RNA bands on agarose gels, revealing that RNA degradation did not occur. A faint background smear is visible, probably

corresponding to mRNA (Fig. 1). The A<sub>260</sub>/280 ratio of RNA extracted by the present method was approximately 2.0, which was comparable to the commercial methods, whereas the A<sub>260</sub>/230 ratio was significantly higher than that of commercial kits. The low A<sub>260</sub>/230 ratio of RNA prepared by commercial kits was accompanied by insoluble RNA pellet, suggesting co-precipitation of polysaccharides (Table 1).

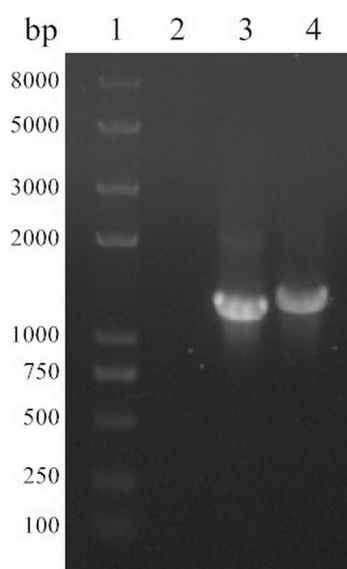
In the new extraction technique, the polysaccharides were removed by following steps: an initial step, the high salts-based extraction buffer was employed to remove most of the polysaccharides. Polysaccharides co-precipitate with nucleic acids in low ionic strength buffers [5]. Moreover, the saturated NaCl solution treatment before organic solvent, further eliminated polysaccharides materials [6]. Phenol/chloroform was a de-proteinization solvent employed to protect RNA from RNase degradation. pH of the phenol-based buffer was acidic which made RNA more stable [7]. Furthermore, the low pH environment allowed efficient separation of RNA and DNA, consequently isolating RNA from DNA and other impurities [8]. To precipitate RNA, LiCl was used. The selective precipitation of LiCl was necessary, maybe because the polysaccharides concentration was still high [9]. High ionic strength buffers combined with LiCl precipitation effectively prevent the co-precipitation of polysaccharides along with RNA [10]. In the present study, two incubation periods, 2 hr and overnight, for LiCl precipitation were examined. Though the overnight precipitation can obtain more RNA, it also gets a lower A<sub>260</sub>/230 (data not shown).

The assessment of RNA integrity is critical first step for downstream molecular study. RIN is a tool for RNA quality assessment [11]. The RIN is from 1 to 10, with 1 being the most degraded profiles and 10 being the most intact. On confirmation of these results of *T. fuciformis* strains, the present method can obtain intact RNA (RIN = 8.9–9.5), which is larger than commercial RNA extraction kits (Table 1). In addition, extracted RNA was amenable to downstream application as evidenced by application through reverse transcriptional PCR (RT-PCR) and quantitative real-time PCR (qRT-PCR). The  $\beta$ -tubulin gene (GenBank accession No. HQ 229921.1) and peroxidase gene (GenBank accession No. GU723632.1) was cloned successfully with the isolated RNA as template following the manufacturer's instructions (TransScript first-strand cDNA synthesis

**Table 1.** Absorbance ratios, RIN value, and RNA yields of *Tremella fuciformis* strains

Methods	Strains	A <sub>260</sub> /280	A <sub>260</sub> /230	RIN	RNA yields ( $\mu\text{g/g}$ FW)
Present protocol	Mycelium M1332	1.85 $\pm$ 0.04	1.58 $\pm$ 0.01	9.3	108 $\pm$ 3.91
Present protocol	Yeast-like cell Y13	2.03 $\pm$ 0.01	1.80 $\pm$ 0.02	8.9	213 $\pm$ 3.32
Present protocol	Yeast-like cell Y32	2.00 $\pm$ 0.01	1.77 $\pm$ 0.04	9.5	135 $\pm$ 6.29
TRIZol reagent	Yeast-like cell Y13	1.97 $\pm$ 0.01	0.78 $\pm$ 0.02	9.0	152 $\pm$ 9.62
RNAiso plus	Yeast-like cell Y13	2.11 $\pm$ 0.01	0.61 $\pm$ 0.03	8.4	146 $\pm$ 7.26

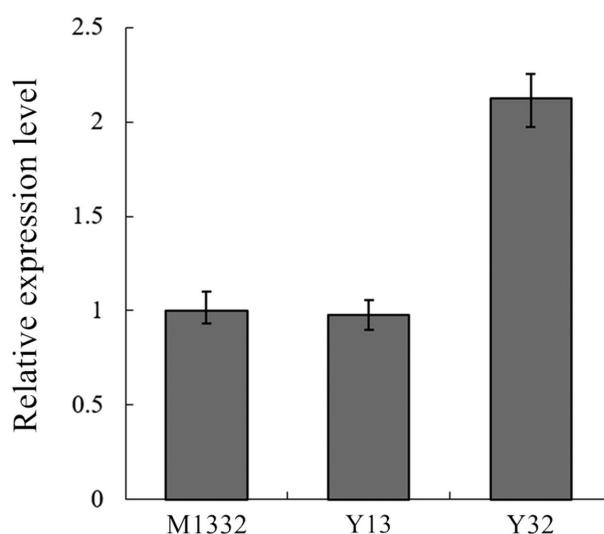
Values are presented as means  $\pm$  SD (n = 3).  
RIN, RNA integrity number.



**Fig. 2.** Electrophoresis of reverse transcriptional PCR products. Lane 1, DNA marker; lane 2, control without reverse transcriptase; lane 3,  $\beta$ -tubulin gene; lane 4, peroxidase gene. The molecular weight of DNA marker (bp) is shown on the left.

supermix; Transgen, Beijing, China) (Fig. 2). F1 (5'-ATGCG-CGAAGTATTTCCTTC-3') and R1 (5'-CTAGTATTCGC-CTTCCTCCTCTCAG-3') were RT-PCR primers for  $\beta$ -tubulin gene and F2 (5'-GGATGGGCCTGATCTACGTC-3') and R2 (5'-GGGTCAAGCCAGATCGAAGC-3') were RT-PCR primers for peroxidase gene. And qRT-PCR was performed according to the manufacturer's protocol (Takara) on the ABI ViiA7 Real-Time PCR System (Applied Biosystems, Foster, CA, USA), using  $\beta$ -tubulin gene as the endogenous control. F3 (5'-GATGACCATTTCTTGCTTC-3') and R3 (5'-GTTCTGACATTTGCTACCG-3') were qRT-PCR primers for  $\beta$ -tubulin gene and F4 (5'-CAGTGGACCGCCAAGCAAA-3') and R4 (5'-TGAACCAGGCTCGGATGAA-3') were qRT-PCR primers for peroxidase gene. Each qRT-PCR reaction was carried out with independent triplicate trials and the expression ratios were calculated according to the  $2^{-\Delta\Delta Ct}$  method [12]. Both  $\beta$ -tubulin and peroxidase gene were successfully amplified in mycelium and yeast-like cells by qRT-PCR, showing Ct values between 17.87 and 20.26 (data not shown). The relative fold differences of the peroxidase gene for *T. fuciformis* mecelium M1332, yeast-like cells Y13 and Y32 were shown in Fig. 3 (normalized to  $\beta$ -tubulin gene).

In summary, the protocol described here is reliable for *T. fuciformis* RNA extraction. Total RNAs from mycelium and yeast-like cells were of high quality, integrity, and yield. Extracted RNA was reliable to downstream applications, which is suitable for investigating molecular events for dimorphism of *T. fuciformis*. It may be used for isolation RNA from other jelly mushrooms or filamentous fungi that are also rich in polysaccharides.



**Fig. 3.** Relative expression levels of the peroxidase gene for *Tremella fuciformis* mycelium M1332, yeast-like cells Y13 and Y32. Data were presented as mean values of three replicates with the corresponding standard deviations.

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