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Case study - *Yeast*

Health

Rapid methods to extract DNA & RNA from Cryptococcus neoformans.

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FEMS Yeast Research. 2001. Vol 1.

Introduction

Extraction of nucleic acids from the pathogenic yeast Cryptococcus neoformans is hampered by a thick and resistant capsule accounting for at least 70% of the whole cellular volume.

This study presents an effective procedure based on mechanical cell breakage using the FastPrep® system to extract RNA from C. neoformans and other capsulated species.

Overview

- **Keywords:** Yeast, DNA, RNA, Extraction, Capsule, Method, Cryptococcus neoformans
- **Aim of the study:** Development of consistent extraction method for DNA & RNA extraction from resistant strains of Cryptococcus neoformans
- **Application:** RNA extraction
- **Sample name:** Cryptococcus neoformans
- **Sample type:** Yeast
- **Material:** FastPrep-24™ instrument, 0.4-0.6 mm glass beads
- **Buffers:** TE buffer, RNA lysing solution (0.5% SDS and 0.5% N-laurylsarcisine)

Protocol and Parameters

Cells were grown in YEPD (yeast extract 1%, peptone 1%, dextrose 2%) for 18h at 25 °C in 500-ml shaken (150 rpm) flasks with a liquid to air ration of 1: 10.

1. Cells from 15 ml overnight culture (approx.10^9) were collected, washed with cold water, resuspended in 200 µl of TE and distributed into two 1.7 ml microcentrifuge tubes.
2. 0.5 ml of glass beads (0.4-0.6 mm), 250 µl of RNA lysing solution, 250 µl 4 M guanidine thiocyanate with 25 mM sodium citrate, pH 7, 0.1 M β-mercaptoethanol, 500 µl phenol, pH 5 and 100µl chloroform/isoamyl alcohol (24 :1) were added to each tube.
3. Both tubes were placed in the FastPrep-24™ instrument and processed in 4 cycles of 40 sec each. Between the cycles samples were placed on ice.
4. After last cycle tubes were removed from the instrument, placed 5 min in ice and spun 10 min at 13,000 x g.
5. The upper phase was transferred to fresh microcentrifuge tube for RNA purification.

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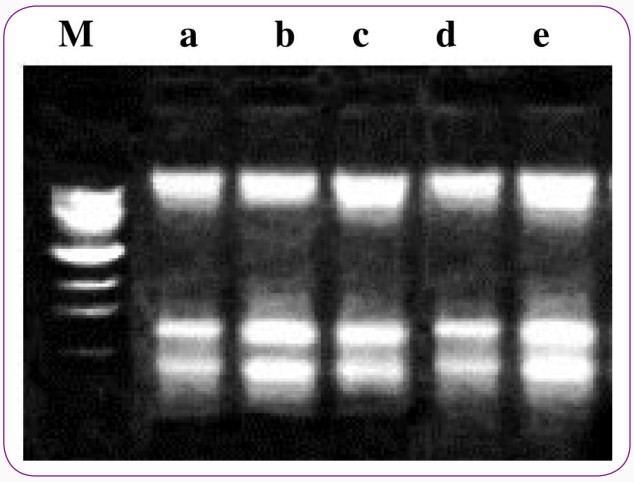
Results

Effective C. neoformans RNA extraction from clinical isolates



Electrophoresis of total cellular RNA extracted from C. neoformans and S. cerevisiae on a 0.8% agarose.

Lane M, 1 kb ladder. Lanes a, b, c and d, C. neoformans RNA extracted from clinical isolates from cerebrospinal fluid of an AIDS patient (a, first episode; b, relapse) and from CBS collection (c, CBS 6995 encapsulated strain; d, CBS 7698 non-capsulated strain). Lane e, S. cerevisiae RNA extracted from the DBVPG 6820 strain.

Conclusion

- RNA purification is accomplished using FastPrep® system and glass beads after a preliminary bead beating treatment
- Yields range around 1 mg RNA from 15 ml overnight culture (10^9 cells)
- RNA appears undegraded, making it suitable for molecular manipulations

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33F0715-1