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RNA extraction from mycotoxigenic filamentous fungi

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CONTEXT

Filamentous fungi cell wall and endogenous RNase activity often hindered the study of the **molecular mechanisms behind secondary metabolites**. **Toxigenic secondary metabolites** affect human health and represent a risk in the food chain system. The conditions in which **mycotoxins** are produced are still not completely understood especially the molecular mechanism that trigger their production.

The extraction of **high quality RNA in good amounts** was therefore critical to further evaluate the conditions in which mycotoxigenic gene clusters are activated. ***Aspergillus flavus*** NRRL 3357 was used to evaluate the RNA extraction protocol. A manual grinding method using mortar and pestle was compared to different bead beating homogenizers using different beads [1].

MATERIAL

- Precellys 24 homogenizer vs Mortar and pestle.
- Precellys kits: CK14, CK28, VK01, VK05 (2mL vials).
- Sample: ~150 mg of mycelium of *A. flavus* NRRL 3357.
- Lysis buffer: 750 µL, either TRIzol or RLT buffer (provided with Qiagen RNeasy plant mini kit) supplemented with β-mercaptoethanol.

PROTOCOL

- Precellys 24: 6500 rpm, 2x25 secs (5 sec break).
- The lysate was centrifuged at +4ºC for 5-10 minutes at 16000 g for an initial homogenization.
- RNA purification: Qiagen RNeasy plant mini kit, QIAcube® robotic workstation.

[1] G.M. Leite, N. Magan, A. Medina. 2012. Comparison of different bead-beating RNA extraction strategies: an optimized method for filamentous fungi. Journal of Microbiological Methods, 88, 413-418.

RESULTS

Significant differences were found between the total RNA amounts isolated using the Precellys 24 homogenizer and the manual system (p-value=0.0072). Furthermore the use of glass beads resulted in a yield around 3 times higher than using the traditional method of the mortar and pestle (Figure 1). The high quality of the RNA as also achieved as an example the electropherogram of a high quality RNA sample extracted using the 0.5 mm glass beads (Figure 2).

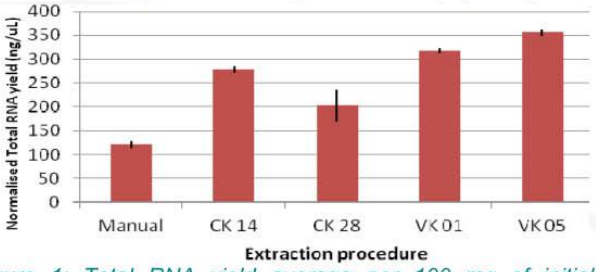

Figure 1: Total RNA yield average per 100 mg of initial biomass and standard deviation comparing different beads with the manual method. Key to treatments: CK – Zirconnium Oxide, VK – Glass; followed by the bead size code 01 – 0.1 mm, 05 – 0.5 mm, 14 – 1.4 mm, 28 – 2.8 mm.

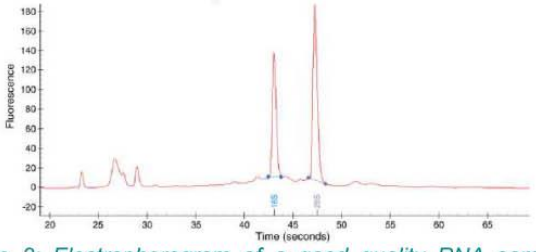

Figure 2: Electropherogram of a good quality RNA sample extracted using Precellys at room temperature. The RQI of this sample is 9.7.

CONCLUSION

The use of the **Precellys 24** with the prefilled **Precellys lysing kit VK05 significantly improved the concentration and the quality** of the **total RNA** extracted from **mycotoxigenic filamentous fungi** while reducing the required amount of mycelium.

The **ease of use** and the **time reduction** aided in the **molecular studies of mycotoxigenic filamentous fungi.**

For more details, please contact precellys@bertin.fr

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