Available online at www.sciencedirect.com
ScienceDirect
Journal of Microbiological Methods 70 (2007) 75–81
Journal of Microbiological Methods
www.elsevier.com/locate/jmicmeth

Comparison of four different methods for extraction of *Stachybotrys chartarum* spore DNA and verification by real-time PCR

J.A. Black *, K.K. Foarde

RTI International, 3040 Cornwallis Road, Research Triangle Park, NC 27709, USA

Received 20 December 2006; received in revised form 17 March 2007; accepted 17 March 2007
Available online 6 April 2007

Abstract

A comparison of four different methods for the extraction of spore DNA from *Stachybotrys chartarum* was conducted. Spore DNA was extracted and purified using either one of three different commercial kits or water. All preparations utilized bead milling. Genomic DNA extracted from 10^1 to 10^7 spores was assessed by both agarose gel electrophoresis and real-time quantitative polymerase chain reaction (qPCR) performed against multi-copy (rRNA) and single-(tubulin) gene targets. The spore isolation technique we employed was verified to be pure by light microscopy. Although all preparatory methods led to successful detection by qPCR, *S. chartarum* spore DNA prepared using the Qiagen Plant kit was notably better over the extraction range.
Published by Elsevier B.V.

Keywords: *Stachybotrys chartarum*; rRNA; Tubulin; qPCR

1. Introduction

It is well known that fungal spores are resistant to lysis and DNA isolation (Martin and Haider, 1971). In addition, there is also increased potential release of PCR inhibitory compounds during extractions. Several studies have been devoted to the development and comparison of different procedures for the extraction of DNA from fungal and bacterial spores (Moller et al., 1992; Gang and Weber, 1995; Loffler et al., 1997; Kutchma et al., 1998; Muller et al., 1998; Kuske et al., 1998; Haugland et al., 1999, 2002). However, these studies have generally utilized high numbers of spores and related absolute quantitation of their yields in terms of the total amount of purified DNA in serially diluted spore extracts. At best, such measurements are only approximates of spore number and limits the sensitivity of the PCR to actually detect and quantify the target organism.

Clearly, a distinctive method for the rapid isolation of quality high molecular weight DNA template from fungi spores regardless of spore number is needed. Although commercial kits are available for DNA isolation from many different types of organisms, they vary widely in the yields of high molecular weight DNA from fungi spores. The unique difficulties in

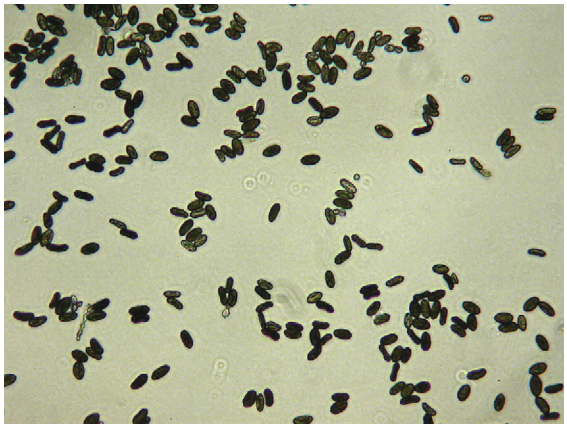


Fig. 1. Light microscopy image of the *Stachybotrys chartarum* spores used for the genomic DNA preparations. It is evident from this 400× image that the majority of the preparation was of intact light dense spores and not of either hyphae or conidiophore.

* Corresponding author. Microbiology and Molecular Biology Department, RTI International, 3040 Cornwallis Road, RTP, NC 27709, USA. Tel.: +1 919 316 3806; fax: +1 919 541 6936.
E-mail address: jab@rti.org (J.A. Black).

0167-7012/$ - see front matter. Published by Elsevier B.V.
doi:10.1016/j.mimet.2007.03.017

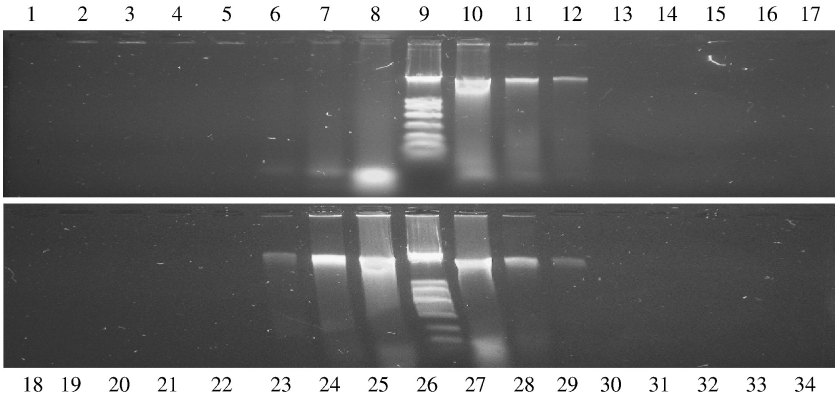


Fig. 2. Agarose gel electrophoresis and SYBR Green I staining of *S. chartarum* spore genomic DNA. Water prepared (lanes 1–8), Promega SV Genomic prepared (lanes 10–17), Qiagen Plant (18–25), and Qiagen Tissue (27–34). Lanes (1) No DNA preparation; (2) 10^1 spores; (3) 10^2 spores; (4) 10^3 spores; (5) 10^4 spores; (6) 10^5 spores; (7) 10^6 spores; (8) 10^7 spores; (9) Ladder; (10) 10^7 spores; (11) 10^6 spores; (12) 10^5 spores; (13) 10^4 spores; (14) 10^3 spores; (15) 10^2 spores; (16) 10^1 spores; (17) No DNA preparation; (18) No DNA preparation; (19) 10^1 spores; (20) 10^2 spores; (21) 10^3 spores; (22) 10^4 spores; (23) 10^5 spores; (24) 10^6 spores; (25) 10^7 spores; (26) Ladder; (27) 10^7 spores; (28) 10^6 spores; (29) 10^5 spores; (30) 10^4 spores; (31) 10^3 spores; (32) 10^2 spores; (33) 10^1 spores; (34) No DNA preparation.

isolating fungi spore DNA was overcome by the experimental procedures described in this report.

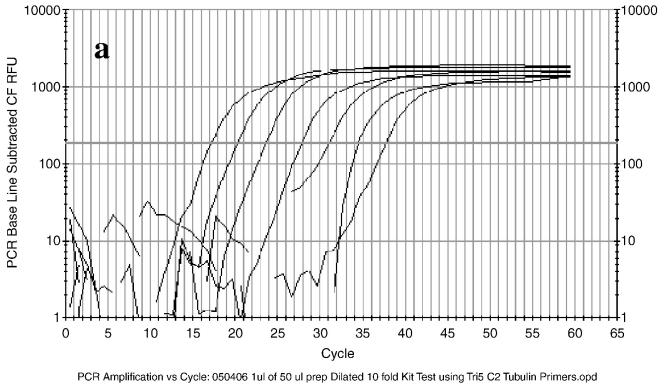
2. Materials and methods

2.1. Reagents and kits

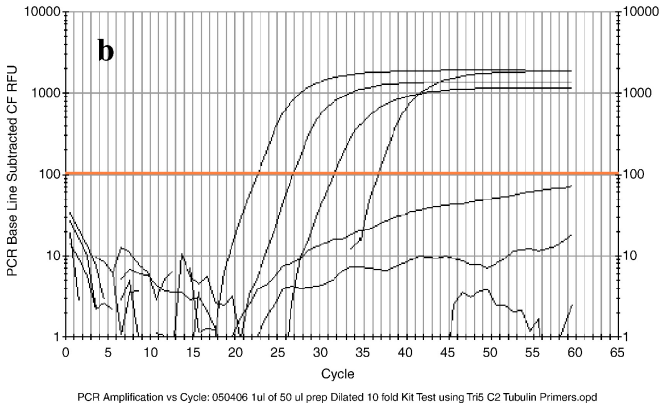
The DNA isolation kits utilized in this study were Promega SV Genomic (Madison, WI), Qiagen Tissue (Valencia, CA), and Qiagen Plant (Valencia, CA), For PCR, the iQ Supermix (BioRad, Hercules, CA) was used.

2.2. Fungi

The test organism selected for this project was an in-house field isolate of *Stachybotrys chartarum* (5111) cultured on 2% Malt Extract Agar (MEA) for 14–21 days at room temperature under incandescent lights.



Threshold Cycle	Identifier
17.9	5111 10^7 spores
20.9	5111 10^6 spores
24.3	5111 10^5 spores
28.5	5111 10^4 spores
31.6	5111 10^3 spores
34.1	5111 10^2 spores
38.2	5111 10^1 spores



Threshold Cycle	Identifier
23.7	5111 10^7 spores
26.7	5111 10^6 spores
31.1	5111 10^5 spores
35.2	5111 10^4 spores
ND	5111 10^3 spores
ND	5111 10^2 spores
ND	5111 10^1 spores

Fig. 3. Real-time detection of *Stachybotrys chartarum* DNA isolated by the Qiagen Plant kit preparation as described in the text. The rRNA gene cluster and tubulin primer sets were used to demonstrate sensitivity with multi-copy (a) and single copy (b). Curves represented by Threshold Cycle (C_T) values for each spore preparation tube are presented in log view. ND indicates no detection by real-time PCR.