Original article

Use of magnetic beads to extract fungal DNA

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Summary

Authors compare two methods of extracting DNA from different fungi: the classic method with phenol/chloroform (P/C) and that with magnetic beads. Both were tested on *Candida albicans* and *Cryptococcus neoformans* var. *neoformans*, belonging to the yeast group and *Microsporum canis*, *M. gypseum*, *Trichophyton rubrum*, *T. interdigitale*, *T. ajelloi*, *Epidermophyton floccosum*, belonging to the dermatophytes group. Extraction products underwent polymerase chain reaction (PCR) fingerprinting with the appropriate primers to point out any disagreement in the genomic profiles. After having determined that the genomic profiles obtained from the DNA extracted from the same strain with the two methods correspond perfectly, the authors concluded that the extraction method with magnetic beads from fungal cells is simpler and quicker than with P/C extraction, greatly facilitating the obtainment of fungal DNA.

Key words: DNA extraction, fungal DNA, magnetic beads.

Introduction

Recent molecular methods for epidemiological subtyping or taxonomic research, such as random amplified polymorphic DNA (RAPD) or restriction fragment length polymorphism (RFLP) and polymerase chain reaction (PCR)-based diagnostic techniques, have determined the need for a rapid and easy method of extracting genomic DNA from different microbial agents and from viruses. However, for fungi the presence of a complex cell wall makes working times longer since an enzymatic and/or mechanical pretreatment which eliminates this structure is necessary; nevertheless it has been possible to extract fungal DNA from fungi both non-pathogenic and pathogenic in man, even if with differentiated methods and not always quick.

In 1989, Morrissey *et al.*[1] have introduced the use of magnetic beads to capture the target DNA hybridized with a probe; these magnetic beads were then successfully used for extracting DNA from various tissues or infectious agents simply and quickly[2–8] allowing to save a noticeable amount of time in comparison with traditional methods, and avoiding the manipulation of harmful substances.

Nevertheless, the first news of the use of this method of extracting DNA from fungi is from Rudi *et al.*[9] and Loeffler *et al.*[10] who in 1997 applied it respectively to fruit bodies and mycelia of various fungi and on *Candida albicans* and *Aspergillus niger*, while Scott *et al.*[11] in 2000 used it for a fungus with agricultural interest, *Claviceps africana*.

We used magnetic beads (Dynabeads DNA Direct System I; Dynal, Oslo, Norway), for extracting DNA from the yeasts *C. albicans*, and *Cryptococcus neoformans* var. *neoformans* and some species of dermatophytes (*Microsporum canis*, *M. gypseum*, *Trichophyton rubrum*, *T. interdigitale*, *T. ajelloi*, *Epidermophyton floccosum*), and we compared it with the classical extraction method with phenol/chloroform (P/C),[12] in order to verify an actual greater speed in obtaining a detectable quantity of PCR-ready DNA.

As for dermatophytes, the DNA was also extracted from 3-day cultures (young colonies, YC) and from strains without typical morphological characteristics (SWTMC), with the aim of speeding up the method as a whole, and from dead dermatophytes to verify the stability of the genomic characteristics after death.[13]

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The DNA extracted with the two methods underwent a PCR with the appropriate primers, in order to compare the genomic profiles obtained and to verify identity.

In fact, on the basis of our previous studies concerning fungi[14, 15] we have established a strict correspondence between species of fungus and genomic profile.

Materials and methods

Candida albicans

Dynabeads DNA extraction. We tested 40 strains isolated from the oral cavity of HIV-positive patients and HIV-negative subjects, by means of the Dynal, partly modified. The yeasts were grown on a minimum medium (MM) (yeast nitrogen base, agar 2%, glucose 2%; pH 7.4) for 24 h at 35 °C, then the fungal growth was taken and washed with 5 ml of SCS (sorbitol 1 mol l^{-1}, trisodium citrate 20 mmol l^{-1}; pH 5.8) centrifuged and resuspended in 200 µl of H_2O (about 10^9 yeast cells ml^{-1}).

About 20 µl of the suspension are incubated with 200 µl of Dynabeads (paramagnetic polystyrene beads in lysis buffer) for 10 min at 65 °C, so as to obtain cell lysis and the adsorption of the released DNA to their surface. This step is followed by magnetic separation of the intact DNA/Dynabeads complex and by subsequent washing with washing buffer, which removes any residual contaminant and eliminates potential PCR inhibitors. The DNA/Dynabeads complex was then resuspended in TE buffer [Tris-HCl 10 mmol l^{-1} (pH 8), ethylenediaminetetraacetic acid (EDTA) 1 mmol l^{-1}] and DNA eluted for 5 min at 65 °C; then it was ready for PCR or to be stored at −20 °C.

Phenol-chloroform DNA extraction. We examined eight (of the preceding 40 ones) strains. The yeasts were grown on MM for 16 h at 35 °C, then the fungal growth (about 10^9 cells ml^{-1}) was taken and washed with 5 ml of SCS and centrifuged at 3000 *g* per 7 min. The sediment was treated with 2 ml of lysing enzyme from *Trichoderma harzianum* (Sigma, St Louis, MO, USA), at the final concentration of 5 mg ml^{-1} in SCS and incubated for 2 h at 35 °C.

After having determined by means of a counting chamber that 70% of the cells were protoplasts, it was centrifuged at 3000 *g* for 5 min, the supernatant was discarded and 2.5 ml of lysis solution was added (pH 8) [Tris-HCl 10 mmol l^{-1}, EDTA 0.1 mol l^{-1}, proteinase K 2 mg ml^{-1}, *N*-laurosylsarcosine (Sigma) 1%]. It was incubated at 35 °C over night. Precipitation with isopropanol and extraction with P/C followed, according to the method described by Varma and Kwon-Chung.[12]

PCR amplification. The PCR was performed with primer A2 (5′-TGGTCGCGGC-3′).[16]

Cryptococcus neoformans var. *neoformans*

Dynabeads DNA extraction. We examined 25 strains. Because of the capsule presence, the procedure was the same used for *C. albicans* in the P/C extraction until protoplasts were obtained. After centrifugation 100 µl of distilled H_2O were added to the sediment and with 20 µl of suspension we proceeded as for Dynabeads DNA extraction of *C. albicans*.

Phenol-chloroform DNA extraction. We examined 90 strains from pathological specimens (Table 1).

The yeast cells were previously deprived of the capsule, then lysed in a solution of EDTA, Tris-HCl, *N*-laurosylsarcosine (Sigma) 1%, proteinase K. The DNA was then extracted by means of P/C, as described for *C. albicans*.

PCR amplification. The PCR was performed with primer $(GACA)_4$.[14]

Dermatophytes

Dynabeads DNA extraction. It was performed on *M. canis*, *M. gypseum*, *T. rubrum*, *T. interdigitale*, *T. ajelloi*, *E. floccosum* for a total of 140 strains.[15] They were grown in Sabouraud glucose agar (SDA) at 25 °C; after 2 weeks a little mycelium was cut from the agar and transferred to Sabouraud glucose broth for 2 more weeks at 25 °C. The superficial mycelial growth was transferred to a mortar, washed with

Table 1 Typology of the examined samples.

Mycetes	Number of strains	Source	Origin
Candida albicans	40	Oral cavity	HIV+ and HIV−
Cryptococcus neoformans	90	CSF (58)[1]	HIV+
		Blood (15)	HIV+
		Urine (8)	HIV+
		Sputum (6)	HIV+
		Glands (1)	HIV+
		Skin (2)	HIV+
Dermatophytes	140		Man, dog, cat (see Ref. 13)

[1]Number of samples/90.

CSF, cerebrospinal fluid.

distilled water and pestled. About 20 µl of pestled mycelium was transferred to an Eppendorf tube and incubated with 200 µl of Dynabeads as previously described.

Young colonies. This test was conducted on 12 of preceding strains belonging to the following species: *M. canis*, *T. mentagrophytes*, *T. interdigitale*, *T. rubrum*, *M. gypseum*, *E. floccosum*. The strains were grown in SDA (Difco, Detroit, MI, USA) at 25 °C; after 3 days a colony (mean diameter 5 mm) was taken and transferred to an Eppendorf tube containing 40 µl of sterile distilled water. The mycelium was homogenized with a manual homogenizer (Micro-Grinder, PBI International, Milan, Italy) for 1 min. Then 400 µl of Dynabeads were added to the homogenized material and the extraction was effected according to the previously described method.

Strains without typical morphological characteristics. We studied eight of preceding strains which had lost their typical morphological characteristics but which had originally been identified as *M. canis*, *T. rubrum*, *T. interdigitale*. Some aerial mycelium was taken and we proceeded to DNA extraction and PCR fingerprinting as described above.

Dead strains. We studied 12 dead strains (DS) that had originally been identified as *M. canis*, *T. mentagrophytes*, *T. interdigitale*, *E. floccosum*. By using a bacteriological spatula some mycelium was scraped out and DNA was extracted as described above.

Phenol-chloroform DNA extraction. This was performed on 10 strains (*M. canis*, *T. interdigitale*, *T. rubrum*) that had been precultivated, as above. The surface mycelium was collected, washed twice with SCS, and pestled. We proceeded to extraction from all the pestled material according to Bowman.[17]

PCR amplification. The PCR was performed with primer $(GACA)_4$.[13, 15]

PCR amplification

Each DNA solution was diluted with TE buffer and its UV-absorption spectrum was assayed using a Beckman DU-64 spectrometer (Beckman Coulter, Fullerton, CA, USA). We estimated the concentration of nucleic acids based on the absorbance of 260 nm. In all the mycetes, the yield of DNA with the two methods was expressed as µg ml^{-1} and the degree of purity was calculated as the ratio between optical density (OD) measured at 260 nm and that measured at 280 nm (OD 260/OD 280).

The PCR was performed with primer $(GACA)_4$. Each sample of genomic DNA was amplified in duplicate in the same PCR and in repeated PCRs at different times.

Statistical analysis

Statistical analysis to study variability between the extraction methods was carried out using the Microsoft Excel 2000 statistical package, calculating mean, SD and by ANOVA.

Results

Table 1 shows the origin and the source of the examined strains. Table 2 shows the strain samples the DNA was extracted from.

The DNA of 237 strains was extracted by means of Dynabeads and that of 108 strains with the P/C method. In all cases, the greatest number of strains tested with one method includes those tested with the other method, with the exception of the dermatophytes YC, SWTMC, DS for which only the Dynabeads test was used.

To verify the expediency of one method rather than another, we compared the concentration and purity (arithmetical average of the values obtained for all the strains tested in each group) of the DNA extracted by means of Dynabeads method with those obtained through the classical extraction with P/C (Table 3).

We observed that with the P/C method a definitely greater concentration of DNA was obtained, 15 times greater for *C. albicans*, about nine times for *Cr. neoformans* and almost three times for the dermatophytes.

Table 2 Samples of the fungi from which the DNA was extracted.

	Number of tested strains	
Mycetes	Dynabeads	Phenol/chloroform
Candida albicans	40	8
Cryptococcus neoformans	25	90
Dermatophytes	140	10
YC	12	
SWTMC	8	
DS	12	
Total	237	108

YC, young colonies; SWTMC, strains without typical morphological characteristics; DS, dead strains.

Table 3 DNA mean yield and purity with the two extraction methods.

	DNA extraction					
	Phenol/chloroform			Dynabeads		
Mycetes	Yield ($\mu g\ ml^{-1}$)	SD	Purity (OD 260/OD 280)	Yield ($\mu g\ ml^{-1}$)	SD	Purity (OD 260/OD 280)
Candida albicans	6684	1710	1.8	443	235	1.3
Cryptococcus neoformans	2022	728	1.4	223	99	1.2
Dermatophytes	945	545	1.4	329	256	1.4

OD, optical density.

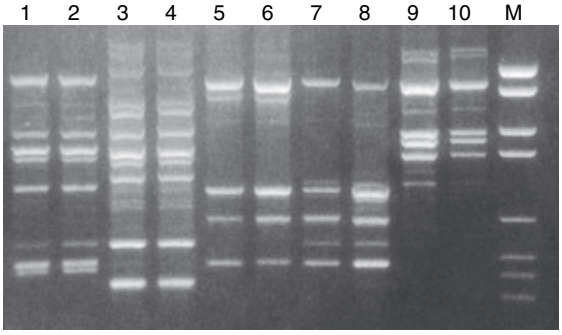

Figure 1 Genomic profiles of *Candida albicans*, *Cryptococcus neoformans* and *Microsporum canis* strains, obtained from the DNA extracted with the two methods [phenol/chloroform (P/C) and Dynabeads]. Lanes: M, DNA size markers; 1–2, *Cr. neoformans* var. *neoformans* serotype A extracted by P/C and Dynabeads; 3–4, *Cr. neoformans* var. *neoformans* serotype D as above; 5–6, *C. albicans* 23554 as above; 7–8, *C. albicans* 23870 as above; 9–10, *M. canis* as above.

The purity grade was greater only for *C. albicans*, while for *Cr. neoformans* and the dermatophytes the value was about the same.

Amplification through PCR with the appropriate primers of the DNA extracted allowed us to compare the quality of the genomic profiles obtained. Figure 1 shows some representative examples of the profiles obtained from the strains tested. The profiles obtained from the DNA extracted with the two methods were perfectly identical: only the *M. canis* profile (lanes 9 and 10) showed some more brightly coloured bands in the DNA extracted by P/C method.

As the cell suspensions of compared strains at start were different for the two extraction methods, we proceeded to compare the DNA mean yield, calculating the quantity of DNA extracted on average from every cell. As regard *C. albicans* there is no significant difference about the yield ($P = 0.9$), whereas as regard *Cr. neoformans* the P/C method obtained a significantly higher yield ($P < 0.001$). As regard dermatophytes, we cannot know the number of cells present in the initial suspension because these are mycelial hyphae, therefore the yield was estimated not at cell but by the weight of the mycelia at start. Also in this case the yield by P/C method was higher ($P = 0.0046$).

Discussion

From the data presented we draw the following conclusions:

The P/C method presents more advantages in comparison with Dynabeads under two aspects: the quantitative/qualitative one, because the rate yield/purity is higher and the economic one, because cost/test is definitely lower in comparison with magnetic beads.

On the contrary, Dynabeads test is advantageous in various aspects. The first one to mention is the ease execution (any operator is able to execute easily the scheduled passages), moreover DNA is obtained in much briefer time (30–40 min vs. 24–25 h with P/C extraction) and without manipulation of harmful substances. There is no need for special laboratory equipment (extractor hood for chemical, supercentrifuge, etc.), but it is enough only the provided kit and a micropipette.

In addition, we must remember the fate of the use of the extracted DNA.

The DNA of the fungal strains tested by us was extracted to undergo PCR fingerprinting so as to obtain genomic profiles to compare for diagnostic and/or epidemiological and/or classification purposes.

Moreover, for this type of research it is not necessary to have great quantities of DNA or it is indispensable to have a high grade of purity, so the yield difference between the two methods is not important.

Thus, we can conclude that the use of magnetic beads greatly facilitates research in the mycological field where PCR-ready DNA must be available, making it possible even in microbiological laboratories with minimum equipment, which allows a certain standardization and homogeneity of the DNA extraction

from both yeast and filamentous DNA which in the past was carried out with the most various and complex methods.

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