American Journal of Biomedical Research, 2023, Vol. 11, No. 1, 7-13
Available online at http://pubs.sciepub.com/ajbr/11/1/2
Published by Science and Education Publishing
DOI:10.12691/ajbr-11-1-2

Implementation of *in-house* Methods for Isolating Fungal DNA of Clinical Samples

David Koffi[1,*], Yayé Yapi Guillaume[2], Francis K. Kouadjo[1,2], Koui S Tossea[1], Andre O. Toure[1], Allico J Djaman[2]

[1]Parasitology and Mycology Department, Institut Pasteur, Abidjan, Côte d'Ivoire
[2]Agrovalorisation Laboratory, University Jean Lorougnon Guédé, Daloa, Cote d'Ivoire
[3]Clinic and Fundamental Biochemistry department, Institut Pasteur, Abidjan, Côte d'Ivoire
*Corresponding author: davidkoffi82@gmail.com

Received January 18, 2023; Revised February 20, 2023; Accepted March 01, 2023

Abstract Over 200 species of fungi are responsible for a variety of infections that can occur in various parts of the human body. There are several phenotypic methods for identifying these fungal elements; however, these approaches have limitations. Molecular methods are now routinely used in well-equipped mycology laboratories. However, the first step in isolating genetic material can often be costly, can suffer from external DNA contamination and some components have toxicity for personnel. The general objective of this work was to identify the best local method for isolating genetic material from fungi based on cost, yield and time-consuming criteria. A total of ten (10) different nucleic acid isolation methods were tested. Those tests using thermal or mechanical shock for cell lysis delivered better quality than those using chemical lysis. Thus, based on our criteria, the best methods for nucleic acid isolation and purification of fungal elements were cetyltrimethylammonium bromide (CTAB) combined with sterilized sea sand (less expensive) and the chelator Chelex® coupled with glass beads (faster).

Keywords: *thermal shock, mechanical shock, fungal species, DNA extraction*

Cite This Article: David Koffi, Yayé Yapi Guillaume, Francis K. Kouadjo, Koui S Tossea, Andre O. Toure, and Allico J Djaman, "Implementation of *in-house* Methods for Isolating Fungal DNA of Clinical Samples." *American Journal of Biomedical Research*, vol. 11, no. 1 (2023): 7-13. doi: 10.12691/ajbr-11-1-2.

1. Introduction

Mycoses are fungal infections caused by microscopic fungi with a filamentous or yeasty appearance. They have been a major public health issue since the 1980s [1]. Routine laboratory identification at the Pasteur Institute of Côte d'Ivoire is done phenotypically. However, these phenotypic methods have limitations. Indeed, they allow the identification of a certain number of yeasts by chromogenic areas and do not identify in an absolute manner through semi-automated or automated methods such as VITEK, MALDI-TOF [2] etc. Considering these limitations, molecular biology approaches present alternative or complementary methods for detecting, identifying and typing fungal pathogens. *Pneumocystis* PCR is on the WHO EDL (Essential Diagnostics List), as a reason for doing the work and many other molecular tests (*Candida* in blood, *Candida auris* on surveillance swabs (OLM Diagnostics®), *Aspergillus* PCR (including species and/or resistance (Pathonostics®, OLM), *Trichophyton* and Mucorales are available.

However, in order to use molecular methods, several important steps are necessary. The first crucial step is the purification of genetic material. This phase consists of three (3) major steps: cell lysis, purification of genetic material, and elution into an appropriate conservation buffer [3]. The lysis step in the case of fungal elements is more complex due to the richness of the cell membrane in polysaccharide [4]. Various commercial kits are available to circumvent this problem but, in some cases, the high cost makes them inaccessible for routine use. In order to avoid commercial kits, various methods have been tested by several research teams. Chemical cell lysis is commonly used, although these products can be toxic to the human body [5,6,7,8]. Beads [8] or enzymatic digestion [9,10] are also viable methods for cell lysis. Membrane or silica matrix applications are also used to recover nucleic acids [11,12].

The general objective of our work was to test various local methods for isolating genetic material from fungal elements in order to identify the best approach according to cost, yield and duration criteria. In this work, tested three methods for cell lysis of fungal material (chemical, thermal or mechanical means) and several approaches for isolation and purification of DNA (Chelex® 100, CTAB and modified Qiagen® cells and tissus DNeasy protocol).

2. Material and Methods

2.1. Fungal Cultures

Single cultures of *Candida albicans* and *Aspergillus fumigatus* were obtained from clinical samples of patients

at the Pasteur Institute of Côte d'Ivoire (Table 1). After freeze preserving in Brain heart broth or Bouillon Coeur cervelle (BCC) + glycerol, they were grown on Sabouraud media solid agar for 2 days for *Candida albicans* and 5 days for *Aspergillus fumigatus*. After culture, mycelium were transferred to a locking microcentrifuge tube with 300µl of sterile Phosphate Bovine Saline (PBS) 1X to obtain 0.5 McF opacity suspension.

2.2. Extraction Methods

2.2.1. Lysis Step

Three different lysis protocols are described here: (i) thermal lysis, (ii) physical lysis, (iii) chemical lysis. For thermal lysis, suspensions were mixed and placed in liquid nitrogen for 30 minutes while thawing every 5 minutes in a liquid water bath at 100°C. For mechanical lysis, suspensions were transferred to a locking microcentrifuge tube containing glass beads or sterilized sea sand. Lysis was achieved by vortexing for 30 min at the highest intensity setting utilizing a Bead-Bug for 1 min 30 sec. For chemical Lysis, 200 µL of suspension were transferred in 200 µL of CTAB buffer (100 mM Tris pH 9; 1.4 M NaCl; 20 mM EDTA; 2% (w/v) hydroxycetyl trimethylammonium bromide (CTAB); 0.2 % (v/v) β-mercaptoethanol) or ATL lysis buffer Qiagen, vortexing then placed in water bath at 65 °C for 30 min.

The lysate obtained from each lysis method was used for nucleic acid purification.

2.2.2. Purification Step

2.2.2.1. Mechanical or Thermal Lytic Methods:

2.2.2.1.1. Phenol/Chloroform/Isoamyl Alcohol Method Modified [13]

To remove residual cellular debris, 250 µL of Phenol/Chloroform/Isoamyl Alcohol solution was added to the lysate and the microtube was centrifuged for 5 minutes at 15000 rpm and the supernatant transferred to a new microtube. Two volumes of absolute ethanol and 1/10 of Sodium acetate 3M were added to the supernatant, and the microtube was centrifuged for 20 min (15000 rpm). DNA was precipitated with 1 mL of 70% ethanol (centrifugation for 20 mn, 15000 rpm). The ethanol was removed and the pellet was dried at room temperature.

2.2.2.1.2. Chelex

To use Chelex protocol, 250 µL of Chelex 5% previously activated by heating to 100°C in a water bath for 5 min, and 20 µl of proteinase K was added to the lysate. The microtube was incubated at 65°C for 30 min. To remove proteins debris, the microtube was spun at 12000 rpm for 2 min. The supernatant was transferred in new microtube and spun at 12000 rpm for 2 min. The supernatant was transferred again in new microtube for storage at -20°C.

2.2.2.1.3. CTAB

This procedure was employed with sterilized sea sand, glass beads and thermal lysates. The purification started by add 800 µL of CTAB buffer previously heated at 65°C for 5 min in the water bath following by vortexing for 20-30 seconds. The microtube was incubate at 65°C for 30 minutes and continuous vortexing every 10 minutes; following by addition of 800 µL of Chloroform/Iso Amyl Alcohol (24:1), vortexing for 30-60 seconds and centrifuged at 13000 rpm for 10 minutes. The supernatant was transferred to a new microtube and mixed with 500 µl isopropanol then incubated at -20°C for 2 hours or overnight. After incubation, the microtube was centrifuged at 13000 rpm for 10 minutes and the pellet was transferred again in new microtube. DNA was washed by adding 200 µl of 70% ethanol to the supernatant and centrifuged at 13000 rpm for 5 minutes. The purification was achieved by removing ethanol and the pellet was dried at room temperature.

2.2.2.1.4. Qiagen Cells and Tissus DNeasy

The lysates were centrifuged at 1880 RPM for 5 min and the supernatant was transferred to a new 2 ml Eppendorf tube. The purification continued following the Qiagen protocol.

Table 1. Summary of DNA extraction methods in this study

Méthods		Quantity	Method of lysis	Purification buffer	Elution buffer	Duration of protocol
1	Phenol/Chloroform/Isoamyl alcohol (P/C/I) (Sofiane, 2010)	0,5 Mc Farland (yeast) 100 mg of mycelium	Chemical	250 µl Phenol/Chloroform/Isoamyl alcohol (P/C/I)	100 µl AE buffer	2h
2	CTAB	0,5 Mc Farland (yeast) 100 mg of mycelium	Chemical	200 µl 2% CTAB buffer (100 mM Tris pH 9; 1.4 M NaCl; 20 mM EDTA; 2% (w/v) CTAB)	100 µl AE buffer	1h10 min
3	CTAB + Freezing/thawing	0,5 Mc Farland (yeast) 100 mg of mycelium	Thermal	800 µL 2% CTAB buffer (100 mM Tris pH 9; 1.4 M NaCl; 20 mM EDTA; 2% (w/v) CTAB)	100 µl AE buffer	4h
4	CTAB + Glass beads		Mechanical			3h
5	CTAB + Sterilized sea sand					3h
6	Chelex + ATL	0,5 Mc Farland (yeast) 100 mg of mycelium	Chemical	200 µl Chelex 5% and 200 µl from buffer ATL Qiagen	---------	50 min
7	Chelex + Glass beads		Mechanical			55 min
8	Chelex + Freezing/thawing		Thermal			2h45 min
9	Qiagen + Freezing/thawing	0,5 Mc Farland (yeast) 100 mg of mycelium	Thermal	200 µl from buffer ATL Qiagen	100 µl AE buffer	1h30 min
10	Qiagen + Glass beads		Mechanical			1h00 min

2.2.2.2. Chemical lytic Methods

2.2.2.2.1. CTAB

Proteins were removed by transferring the lysate supernatant to CTAB in a new 1.5 ml Eppendorf tube after extraction with 200 µl of chloroform mixed by inversion and centrifuged at 12000 RPM for 5 min. 200 µl of isopropanol was added and mixed via inversion, followed by centrifugation at 12,000 RPM for 15 min to separate and wash the DNA from the CTAB Buffer. The DNA was precipitated by 200 µl of 70% ethanol by centrifugation at 12000 RPM for 5 min. The ethanol was removed and the extract was left at room temperature to dry the pellet.

2.2.2.2.2. Chelex

To the lysate with ATL Qiagen, 200 µl of Chelex 5% previously activated by heating to 100 °C in a water bath for 5 min was added. Next, the mixture was heated to 100 °C to perform cell lysis. 20 µL of proteinase K was added and incubated at 65 °C for 30 min to activate the enzyme and lyse the proteins. Residual proteins were removed by centrifugation at 12000 RPM for 2 min. The remaining elements were removed by centrifuging the supernatant for 2 min at 12000 RPM.

2.2.3. Elution Step

All purified products were eluated in Qiagen AE buffer with the exception of the DNA isolated using Chelex (products obtained after purification were stored directly at -20 °C as nucleic acid extract).

Extraction methods are summarized in Table a.

The origins of the different isolates and strains used in this study are listed in the table below

Table a. Isolates and strains fungi used

Numbering	Coding	Species	Provenance
A	20062	*Candida albicans*	Ear pus
B	18792	*Candida albicans*	Vaginal swab
E	ATCC6258	*Candida krusei*	Reference strain
C	20081	*Aspergillus fumigatus*	Corneal abscess

2.3. Amplification by Conventional PCR

The PCR solutions were prepared with purified template; and each target was amplified according the appropriate programs.

Strip size was evaluated by a visual reading of 8 µl on a 1% agarose gel for 45 minutes at 100V.

The table below shows the different volumes to be taken of each reagent and the necessary concentrations of enzyme and primer in the reaction medium.

Table b. Mix preparation

	Volume/Sample (µL)	Final concentration (mM)
H_2O	14	-
Primer F (10µM)	0,5	0,25
Primer R (10µM)	0,5	0,25
5x FIREPol Master Mix	5	1x
Total mix	20	-
DNA	5	-
Total volume	25	-

Table c. Primer Sequences, target genes and product sizes (Větrovský *et al*., 2020; Desh *et al*., 2006; Lee *et al*., 2004)

Gene name	Sequence	*Candida albicans*	*Aspergillus fumigatus*	*Candida krusei*
Cd1_F	TCCGTAGGTGAACCTGCGG	218 bp	235 bp	218 bp
Cd1_R	GCTGCGTTCTTCATCGATGC	253bp	259 bp	326 bp
Cd2_F	GCATCGATGAAGAACGCAGC	536bp	599bp	
Cd2_R	TCCTCCGCTTATTGATATGC		--	536 bp
ERG11-ORF-F	GAAAGGGAATTCAATCG	1745 bp	--	

Table d. Conventional PCR Amplification Programs by Primer

	PCR-Cd1-F_Cd1-R & Cd1-F_Cd2-R			PCR-Cd2-F_Cd2-R			Optimized programs					
							Program 1			Program 2		
Steps	T°C	Time	Cycles	T°C	Time	Cycles	T°C	Time	Cycles	T°C	Time	Cycles
Initial denaturation	95°C	10 min	1x	95°C	10 min	1x	95°C	10 min	1x	95°C	10 min	1x
Denaturation	95°C	30 s	35x	95°C	30 s	35x	95°C	30 s	40x	95°C	30 s	40x
Hybridization	58°C	30 s	35x	56°C	30 s	35x	58°C	30s	40x	58°C	1 min	40x
Initial elongation	72°C	1 min	35x	72°C	1 min	35x	72°C	1 min	40x	72°C	2 min	40x
Final elongation	72°C	5 min	1x	72°C	5 min	1x	72°C	7 min	1x	72°C	7 min	1x

The different times required for each amplification step for each primer pair are listed in the table above.

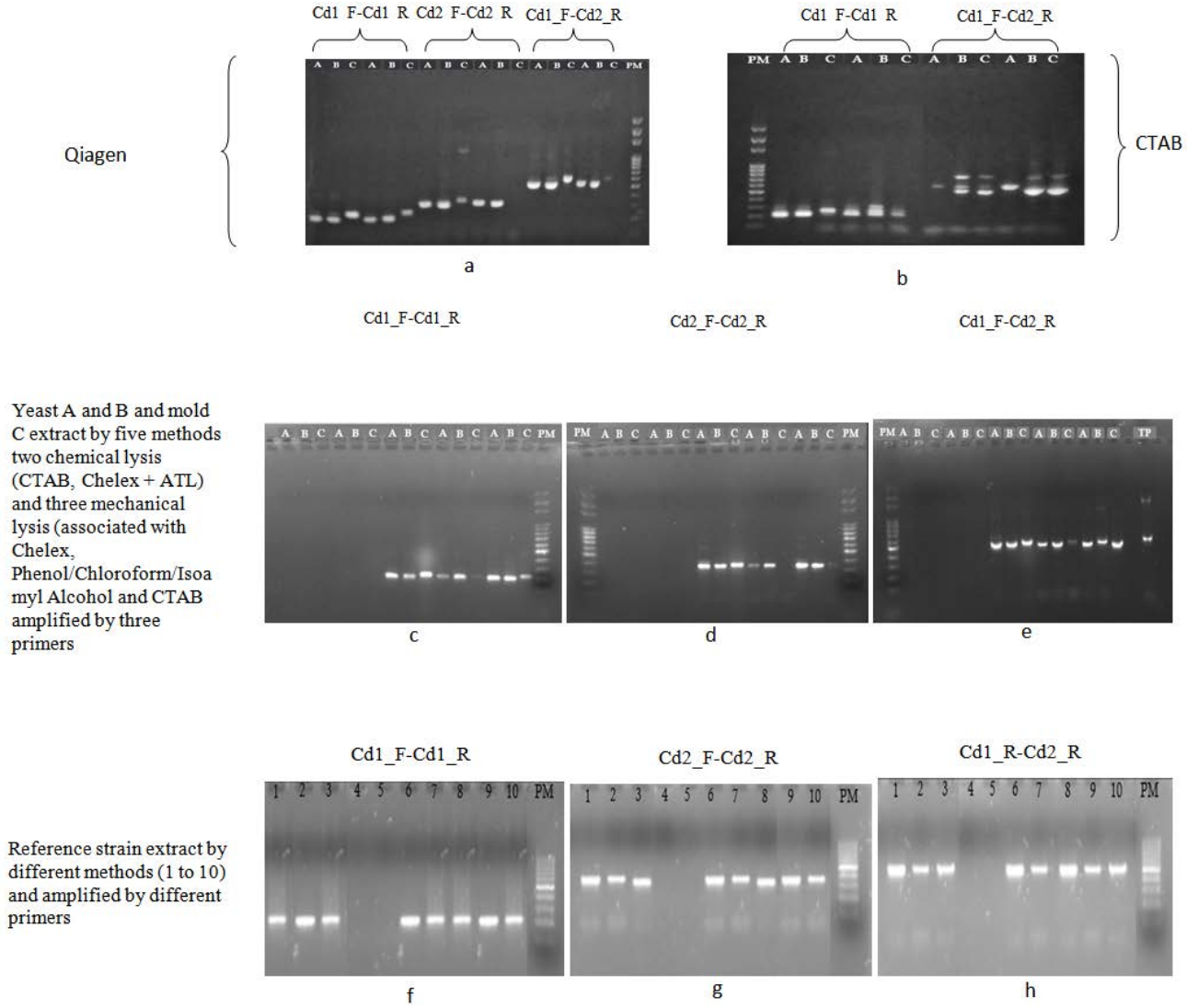

Figure 1. gel electrophoresis prepared at 1% TBE 1X for DNA amplification with ITS primers and a molecular weight marker PM 100. (a) Qiagen amplification of the extracts with the primers Cd1-F_Cd1-R (strips 1st A to 2nd C), Cd2-F_Cd2-R (strips 3rd A to 4th C) and Cd1-F_Cd2-R (strips 5th A to 6th C) respectively, wherein strips first A, B, C, third A, B, C and the fifth A, B, C are derived from the mechanical lysis method and strips second A, B, C, fourth A, B, Cand sixth A, B, C are derived from the thermal lysis method ; (b) amplification of the extracts in CTAB with the primers Cd1-F_Cd1-R (1st and 2nd strips A to C) and Cd1-F_Cd2-R (3rd and 4th strips A to C) respectively, wherein 1st strip A, B, Cand 3rd strip A, B, C are derived from the mechanical lysis method and 2nd strip A, B,C and 4th strip A, B, C are derived from the thermal lysis method; (c), (d) and (e) amplification of the extracts with the primers Cd1-F_Cd1-R (a), Cd2-F_Cd2-R (b) and Cd1-F_Cd2-R (b) respectively with Chelex, CTAB and Phenol/Chloroform/Isoamyl Alcohol wherein the six first bands are derived from chemical lysis methods (CTAB (1st A to C) and Chelex + ATL (2nd A to C)) then nine last bands are successively using mechanical lysis associated with Chelex (3rd A to C), Phenol/Chloroform/Isoamyl Alcohol (4th A to C) and CTAB (fifth A to C). (From strain ATCC6258 the amplification of DNA extracts with ITS primers and a PM 100 molecular weight marker. (f), (g) and (h) amplification of the extracts with the primers Cd1-F_Cd1-R (f), Cd2-F_Cd2-R (g) and Cd1-F_Cd2-R (h), respectively, to ten methods wherein bands 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 are successively derived from methods 1, 3, 4, 2, 6, 5, 7, 8, 9 and 10)

3. Results

A total of ten (10) fungal DNA extraction methods were tested: four (4) which included mechanical tests, four (4) which included thermal tests, and two (2) which based on chemical products for lysis of fungal cells (Table 1). The PCR results were compared between the types of methods (lyses) of the different protocols. Figure 1 shows the results obtained with the identification primers Cd1-F/Cd1-R, Cd2-F/Cd2-R, Cd1-F/Cd2-R for all methods. It shows the size of the bands (desired gene) for the different samples (strains or isolates).

Mechanical lysis of cells on the different isolates and strains was effective for all methods (Figure 1a (1st A-C, 3rd A-C and 5th A-C) Qiagen + Glass beads; 1b (A, B and C) CTAB + Glass beads; 1c, 1d and 1e ((3rd A-C), (4th A-C) and (5th A-C) for Chelex + Glass beads, PCI + Glass beads and CTAB + Glass beads respectively); 1f, 1g and 1h (7 and 10) corresponding to each method for the extract from the reference strain) except for the CTAB + Glass beads method in Figures 1b (3rd A to C) and 1f, 1g and 1h (4) and CTAB + Sterile sea sand in Figures 1f, 1g and 1h (5). The extracts show a double band for extracts of the 3rd B and C due to the presence of other species in the extracts and a low band amplitude for extract 7 due to the presence of RNA. Figure 1.c and Figure 1.e show a low amplitude at extract of the 4th C and Figure 1d show no band for the same extract from the mechanical lysis + PCI method. Thermal lysis shows a weak band with sample 15 with primers Cd1-F_Cd2-R in the Qiagen + Freezing/thawing method (Figure 1a (2nd A-C, 4th A-C and 6th A-C)) due to the presence of RNA. Figure 1.b shows

the presence of the gene of interest in samples 2nd B, 3rd B, C, and 4th B and C with the CTAB + Freezing/thawing method with the presence of other species in the samples. Regarding the chemical cell lysis methods, there is an absence of amplification bands for the extracts of the different chemical methods CTAB (1st A to C) and Chelex + Qiagen ATL lysis buffer (2nd A to C) in images c, d and e and at the level of extracts 2nd B and C as shown in images f, g and h in Figure 1 for the same methods. These samples treated with the chemical extraction methods, were not amplified (Figure 1). These results show that extraction methods involving mechanical lysis concentrate DNA better than extraction methods involving thermal lysis and chemical extraction methods.

Figure 2 shows the results of the amplification of extracts with ERG11-ORF primers with the program according to Lee *et al*. and the optimized program 1 (Cd1_F-Cd1_R). The optimized *program 1* amplifies the extracts better than the program according to Lee *et al*. which amplifies only for one sample (Figure 2). Amplification of extracts (i) according to the program of Lee et al (2004) with the appearance of a band for extract A from the mechanical lysis method combined with Qiagen; no band for extracts B, A and B from the mechanical lysis methods combined with CTAB (B) and thermal lysis methods combined with CTAB (B) and Qiagen (A) respectively. (j) amplification of the extracts according to optimized program 1 with primers Cd1-F_Cd1-R of the extracts from the mechanical lysis methods combined with Qiagen (2nd A) and CTAB (1st A) and from the thermal lysis methods combined with Qiagen (2nd B) and CTAB (1st B). The same extracts were amplified with ERG11-ORF primers with band formation as for the extracts from Qiagen combined mechanical lysis (2) and Qiagen combined thermal lysis (4).

Amplification with ERG11-ORF primers resulted in bands with program 1 (image k) and with program 2 (image l) only with yeast.

Two types of fungi were used in this study: yeasts and molds. These samples were classified into two groups, the group of isolates (species isolated in the laboratory) which are a yeast of the genus *Candida albicans* and a mold of the genus *Aspergillus fumigatus* and a reference strain of the genus ATCC6258 (Table a).

The time required for nucleic acid extraction varied and the protocol duration ranged from 55 minutes for the Chelex ATL method to 4 hours for the CTAB freezing/thawing method (Table 1). Data for the amplification of extracts with the different primers are provided in Table b (supplementary data: mix preparation) and Table d (supplementary data: design of amplification programs).

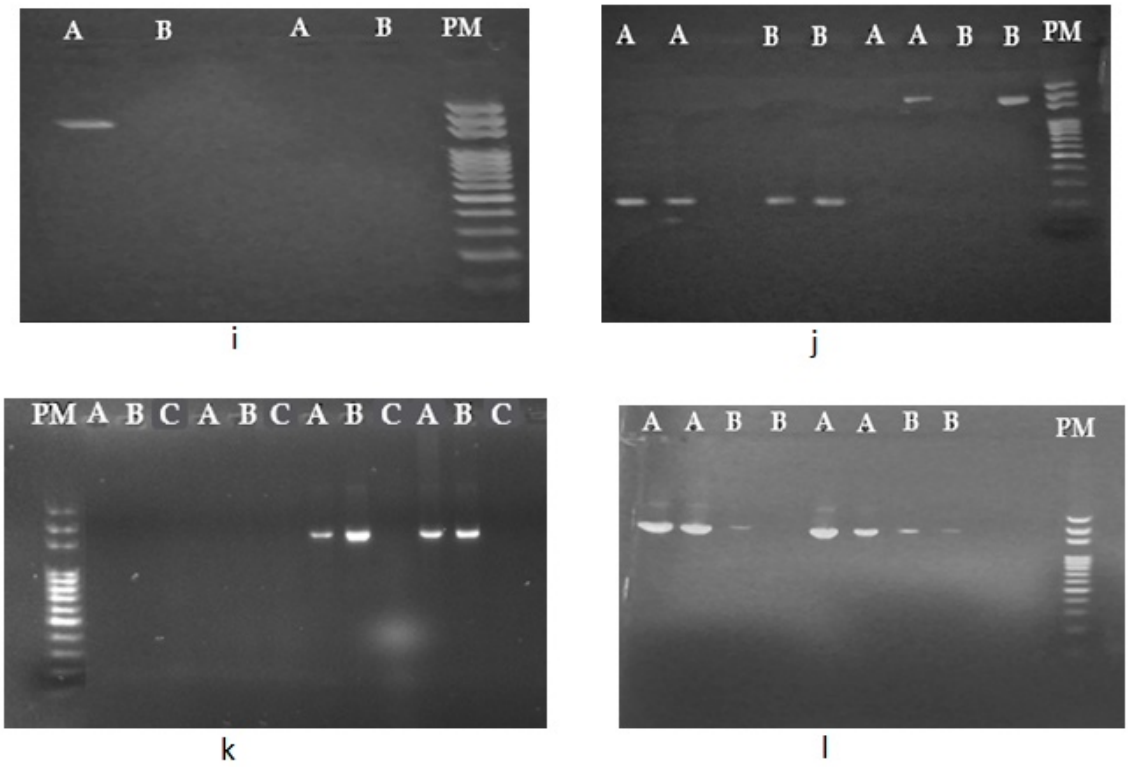

Figure 2. Agarose gels prepared at 1% in TBE 1X for the amplification of extracts with ERG11-ORF primers and a molecular weight marker PM 100. Amplification of extracts (i) according to the program of Lee et al (2004) with the appearance of a band for extract A from the mechanical lysis method combined with Qiagen; no band for extracts B, A and B from the mechanical lysis methods combined with CTAB (B) and thermal lysis methods combined with CTAB (B) and Qiagen (A) respectively. (j) amplification of the extracts according to optimized program 1 with primers Cd1-F_Cd1-R of the extracts from the mechanical lysis methods combined with Qiagen (2nd A) and CTAB (1st A) and from the thermal lysis methods combined with Qiagen (2nd B) and CTAB (1st B). The same extracts were amplified with ERG11-ORF primers with band formation as for the extracts from Qiagen combined mechanical lysis (2) and Qiagen combined thermal lysis (4). Amplification of extracts with ERG11 primers according to the optimized program 2. All the extracts from four methods (chemical lysis (CTAB (1st A to C), Chelex + ATL (2nd A to C); mechanical lysis associated with Chelex (3rd A to C) and thermal lysis associated with Chelex (4th A to C)) were amplified (k). Extracts A and B of yeasts respectively A and B from mechanical lysis methods associated with Qiagen (1st A and 3rd A) and CTAB (2nd A and 4th A) and then from thermal lysis methods associated with Qiagen (1st B and 3rd B) and CTAB (2nd B and 4th B) (l)

4. Discussion

Nucleic acid extraction is an essential step in a molecular biology study. The amplification by conventional PCR of the resulting extracts not only makes it possible to assess the quantity and quality of the DNA in the medium but also the characterization of the species involved in the fungal pathology in order to allow its complete identification [3]. However, the rigidity of the fungal cell membrane due to its high polysaccharide content requires efficient means to extract gDNA [4]. Mechanical lysis methods from glass beads, sea sand sterilized and thermally freeze/thawed in this study has shown efficiency in interrupting and isolating the genetic material. Yamada and *al*. [13] showed that the use of mechanical methods by grinding the fungal cell made it possible to break the cell membrane, and Kanshin and *al.* [14] showed that the sudden change in extreme temperatures caused the yeast cells to rupture. Post-purification amplicons of these lysates by different purification protocols demonstrates the quality of the extracts from these different lysis methods (Figure 1). Avolio and *al*, [15], Rozales and *al*, [16] and Tachikawa and *al*, [17] showed that amplification of extracts by PCR is an indicator of both quality and sufficient quantities of nucleic acids. This confirms the purity and quality of the different extracts of our protocols. Also, El-Kirat [18] highlighted the denaturing and inhibitory action of toxic chemicals or buffer such as the Phenol/Chloroform/Isoamyl Alcohol buffer on DNA and PCR, which could be an inhibiter of PCR in our case as shown in Figure 1. Chemicals such as Phenol and Chloroform are hazardous to health. We have not amplified samples with the chemical method because chemical products used to lysate the membrane of the fungal cells had no effect on the fungal cells after purification of the lysates (Figure 1). According to Vingataramin [3], the efficiency of the cell lysis method allows the concentration of DNA which is critical to obtain sufficient DNA for PCR amplification. However, Abdel-Latif & Osman [19] showed that with the application of a product such as CTAB, it was possible to lyse the fungal cells and obtain a sufficient quantity and a better quality of DNA that could be amplified by PCR. The amplification of the extracts by the different primers confirmed the high quality of DNA products after purification. The sizes of the observed amplification bands indicate the species identity and the accuracy of the primer pairs highlighted here for ITS identification primers (Cd1, Cd2 and Cd1-Cd2). This result partially confirms the identity of the species used in our study. Amplification programs for the identification primers would be suitable for the primers since they allowed the amplification of the extracts.

Amplification of DNA from yeast strains by the ERG11-specific primers of *Candida albicans* through the amplification program of Lee and *al*, [20] did not yield any amplification bands (Figure 2 image i). Whereas, amplification of the same DNA products showed amplification products with the optimized program 1 (Figure 2 image K). Similarly, amplification of all extracts from five methods indicated presence of DNA target only in DNA purified from yeast samples (Figure 2 image l). This shows that ERG11-ORF primers are very specific to *Candida albicans*. Amplifications programs optimized for the amplification of specificity primers would be more suitable for the amplification of these primers. The amplification of the reference strain ATCC6258 confirmed the efficiency of different cell lysis and purification methods. Extraction protocol durations range from 55 minutes to 240 minutes. Dilhari and *al*, [21] have described long protocols ranging from 25 minutes to 11 hours. Long durations of those protocols, costs or toxicity of some products may limit their routine use, but the efficacy of some is worthwhile.

5. Conclusion

Extraction of gDNA from fungal species is difficult and requires the use of sufficiently effective means to break down the cell wall. We have modified, adapted, developed and compared gDNA extraction methods on two groups of fungal species. The best method was based on Chelex combined with glass beads that uses proteinase K because it is less durable and allows extraction of a sufficient amount of gDNA. The lowest cost method was CTAB with sterilized sea sand for cell lysis.

Acknowledgements

We are grateful to John G. Gibbons (University of Massuchetts, US) and David D Denning (University of Mancherster, CEO GAFFI, Global action Fund for Fungal infections) for critical reading and helpful comment of the manuscript. Many thanks to the staff of Molecular Biology Plateform in Pasteur Institute of Cote d'Ivoire for the help and the availability of equipment.

References

[1] Guillot, J., & and Dannaoui E., *La résistance aux antifongiques : importance en médecine humaine et vétérinaire.* Bulletin de l'Académie Vétérinaire de France, 2016.

[2] Cassagne, C., Normand, A. C., L'Ollivier, C., Ranque, S., and Piarroux, R. *Performance of MALDI-TOF MS platforms for fungal identification.* Mycoses, 2016. 59(11): p. 678-690.

[3] Vingataramin, L., *Dépistage des infections du tractus urinaire par méthode moléculaire.* Http://Www.Editorialmanager.Com/Bt/ViewLetter.Asp?Id=325033&1sid={E8... 2014-12-15, 125p., 2014.

[4] Garnaud, C., & and Cornet M., *Membrane et paroi fongiques : des rôles clés dans la résistance aux antifongiques.* Revue Francophone Des Laboratoires, 2020. 519: p. 50–58.

[5] Paterson, A.H., Brubaker C.L., &, and Wendel J.F., *A rapid method for extraction of cotton (Gossypium spp.) genomic DNA suitable for RFLP or PCR analysis.* Plant Molecular Biology Reporter, 1993. 11(2): p. 122-127.

[6] Susanna, H., Kenneth P.R. and Halyna F., *Rapid isolation of yeast genomic DNA: Bust n' Grab*. BMC Biotechnology, 2004. 4(1): p. 1-6.

[7] Cheng, H.R. and Jiang N., *Extremely rapid extraction of DNA from bacteria and yeasts.* Biotechnol Lett, 2006. 28(1): p. 55-9.

[8] Chavasco, J. K., Paula, C. R., Hirata, M. H., Aleva, N. A., De Melo, C. E., Gambale, W., Ruiz, L. D. S., and Franco, M. C. *Molecular identification of Candida dubliniensis isolated from*

oral lesions of HIV-positive and HIV-negative patients in Sao Paulo, Brazil. Rev Inst Med Trop Sao Paulo, 2006. 48(1): p. 21-6.

[9] Borman, A. M., Linton, C. J., Miles, S. J. and Johnson, E. M. *Molecular identification of pathogenic fungi.* J Antimicrob Chemother, 2008. 61 Suppl 1: p. i7-12.

[10] Jin, J., Lee Y.K., and Wickes B.L., *Simple chemical extraction method for DNA isolation from Aspergillus fumigatus and other Aspergillus species.* J Clin Microbiol, 2004. 42(9): p. 4293-6.

[11] Hotzel, H., Müller W., and Sachse K., *Recovery and characterization of residual DNA from beer as a prerequisite for the detection of genetically modified ingredients.* European Food Research and Technology, 1999. 209(3–4): p. 192–196.

[12] Galán, A., Veses, V., Murgui, A., Casanova, M. and Martínez, J. P. *Rapid PCR-based test for identifying Candida albicans by using primers derived from the pH-regulated KER1 gene.* FEMS Yeast Res, 2006. 6(7): p. 1094-100.

[13] Yamada, Y., Makimura, K., Merhendi, H., Ueda, K., Nishiyama, Y., Yamaguchi, H. and Osumi, M. *Comparison of different methods for extraction of mitochondrial DNA from human pathogenic yeasts.* Jpn J Infect Dis, 2002. 55(4): p. 122-5.

[14] Kanshin, E., Kubiniok, P., Thattikota, Y., D'Amours, D., and Thibault, P. *Phosphoproteome dynamics of Saccharomyces cerevisiae under heat shock and cold stress.* Mol Syst Biol, 2015. 11(6): p. 813.

[15] Avolio, M., Diamante, P., Modolo, M. L., De Rosa, R., Stano, P. and Camporese, A. *Direct molecular detection of pathogens in blood as specific rule-In diagnostic biomarker in patients with presumed sepsis: Our experience on a heterogeneous cohort of patients with signs of infective systemic inflammatory response syndrome.* Shock, 2014. 42(2): p. 86-92.

[16] Rozales, F. P., Machado, A. B. M. P., De Paris, F., Zavascki, A. P. and Barth, A. L. *PCR to detect Mycobacterium tuberculosis in respiratory tract samples: evaluation of clinical data.* Epidemiol Infect, 2014. 142(7): p. 1517-23.

[17] Tachikawa, R., Tomii, K., Seo, R., Nagata, K., Otsuka, K., Nakagawa, A., Otsuka, K., Hashimoto, H., Watanabe, K. and Shimizu, N. *Detection of herpes viruses by multiplex and real-time polymerase chain reaction in bronchoalveolar lavage fluid of patients with acute lung injury or acute respiratory distress syndrome.* Respiration, 2014. 87(4): p. 279-86.

[18] El-Kirat, C.S., *Développement d' outils cellulaires et moléculaires pour l' étude des interactions Candida – phagocytes ; Application à la caractérisation du gène OLE2 codant une désaturase chez C. lusitaniae.* Thèse de Doctorat En Microbiologie à l'université Bordaux 2, France., 2010.

[19] Abdel-Latif, A. and Osman G., *Comparison of three genomic DNA extraction methods to obtain high DNA quality from maize.* Plant Methods, 2017. 13: p. 1.

[20] Lee, M., Williams, L. E., Warnock, D. W. and Arthington-skaggs, B. A. *Drug resistance genes and trailing growth in Candida albicans isolates.* J Antimicrob Chemother, 2004. 53(2): p. 217-24.

[21] Dilhari, A., Sampath, A., Gunasekara, C., Fernando, N., Weerasekara, D., Sissons, C., McBain, A. and Weerasekera, M. *Evaluation of the impact of six different DNA extraction methods for the representation of the microbial community associated with human chronic wound infections using a gel-based DNA profiling method.* AMB Express, 2017. **7**(1): p. 179.