RESEARCH ARTICLE

Development and evaluation of loop-mediated isothermal amplification (LAMP) for the rapid diagnosis of *Penicillium marneffei* in archived tissue samples

Jiufeng Sun[1], Xiqing Li[1], Hanxiang Zeng[1], Zhi Xie[1], Changming Lu[1], Liyan Xi[1] & Gert S. de Hoog[2,3,4]

[1]Department of Dermatology, The Second Affiliated Hospital, Sun Yat-Sen University, Guangzhou, China; [2]CBS – KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; [3]Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, The Netherlands; and [4]Peking University Health Science Center, Research Center for Medical Mycology, Beijing, China

Correspondence: Liyan Xi, Department of Dermatology, The Second Affiliated Hospital, Sun Yat-Sen University, 107 West Yanjiang Rd, Guangzhou 510120, China. Tel.:/fax: +86 20 8133 2289; e-mail: xiliyan@mail.sysu.edu.cn

Received 5 November 2009; revised 18 December 2009; accepted 18 December 2009. Final version published online 26 January 2010.

DOI:10.1111/j.1574-695X.2009.00647.x

Editor: Jacques Schrenzel

Keywords
Penicillium marneffei; penicilliosis; rapid diagnosis; LAMP.

Abstract

Penicillium marneffei is the etiologic agent of a severe systemic disease in immunocompromised hosts in Southeast Asia. In the present study, a novel method, known as loop-mediated isothermal amplification (LAMP), is described for the rapid and specific detection of the species, using a primer set derived from the internal transcribed spacer (ITS) region of the rRNA gene. Amplification products can be detected macroscopically by visual inspection in vials using SYBR Green I as well as by electrophoresis on agarose gel. The LAMP assay resulted in specific amplification of *P. marneffei* ITS using pure cultures after a 1-h reaction at 65 °C in a water bath; no cross-reactivity with other fungi including other biverticillate penicillia was observed. The detectable DNA limit was two copies. In addition, specific amplification was achieved using paraffin wax-embedded tissue samples from patients with penicilliosis marneffei and tissue samples from bamboo rats. The method provides a powerful tool for rapid diagnostics in the clinical lab, and has potential for use in ecological studies.

Introduction

Penicillium marneffei is the agent of a life-threatening systemic mycosis known as penicilliosis marneffei, occurring in patients infected with HIV in Southeast Asia (Supparatpinyo *et al.*, 1994; Wong *et al.*, 1998; Liyan *et al.*, 2004) and now recognized as an AIDS-defining disease (Lee, 2008). Cases were particularly frequent in endemic zones of northern Thailand (Watanabe *et al.*, 2008), but the disease has also been observed in China (Fisher *et al.*, 2005). Since the first reported Chinese case in 1985 (Wei, 1985), there has been a drastic increase in the incidence of the infection, concomitant with the emergence of the AIDS pandemic. More than 100 cases of AIDS with penicilliosis marneffei were reported between 2003 and 2006 in a single hospital in Guangzhou (Linghua Li & Weiping, 2008). Clinical diagnosis may be hampered by the fact that major manifestations of the mycosis in HIV-infected patients are not specific for *P. marneffei*. As a result, many patients do not receive timely and appropriate antifungal treatment, and their prognosis is poor. Traditionally, penicilliosis marneffei is diagnosed by a microscopic observation of fungal fission yeast cells in alveolar macrophages and by culturing the etiologic agent. These procedures may be time-consuming (Ukarapol *et al.*, 1998; Mo *et al.*, 2002), and there is a need for experimental diagnostic methods. Serological diagnosis (Panichakul *et al.*, 2002) is tedious because it requires paired, acute- and convalescent-phase sera, and the results may be influenced by contamination or cross-reaction. Several molecular methods have been proposed, such as nested or semi-nested PCR (LoBuglio & Taylor, 1995; Vanittanakom *et al.*, 2002; Prariyachatigul *et al.*, 2003), PCR-enzyme immunoassays (Lindsley *et al.*, 2001) and PCR hybridization (Vanittanakom *et al.*, 1998). All have been developed on the basis of cultured material, and require a fully equipped molecular laboratory. Thus, there is still a need for a rapid and simple technique that is able to deliver an unambiguous identification within a single day.

Loop-mediated isothermal amplification (LAMP) was introduced for the detection of hepatitis B virus DNA by Notomi *et al.* (2000). This novel technique is able to amplify DNA with high specificity, efficiency and rapidity under

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isothermal conditions. The assay is based on the use of Bst DNA polymerase, performing autocycling strand displacement DNA synthesis using a set of four or six specially designed primers that recognize six or eight distinct sequences on the target DNA. The cycling reactions result in the accumulation of 10^9- to 10^{10}-fold amplification of the target in less than an hour. Amplification products can be detected easily by visual assessment of turbidity in Eppendorf vials or by electrophoresis. The sensitivity of LAMP does not appear to be affected by the presence of nontarget DNA in samples, and there is no interference by known PCR inhibitors such as blood, serum, plasma or heparin (Notomi *et al.*, 2000; Enosawa *et al.*, 2003; Poon *et al.*, 2005). These properties of high specificity, selectivity, simplicity and speed made LAMP attractive for the diagnosis of bacteria (Iwamoto *et al.*, 2003; Yoshida *et al.*, 2005; Aoi *et al.*, 2006), viruses (Poon *et al.*, 2004; Hagiwara *et al.*, 2007; Cai *et al.*, 2008) and parasites (Ikadai *et al.*, 2004; Iseki *et al.*, 2007). However, very few papers have appeared on the use of LAMP with fungi (Endo *et al.*, 2004; Ohori *et al.*, 2006; Inacio *et al.*, 2008). We recently developed a protocol for LAMP detection for *Fonsecaea* agents of chromoblastomycosis (Sun, 2009). In the present study, we introduce LAMP diagnostics for *P. marneffei* in paraffin wax-embedded human tissue and in bamboo rat tissue samples.

Materials and methods

Strains and biopsy specimens

Forty strains of *P. marneffei* isolated from human patients and 46 reference strains used in this study are listed in Table 1. All

Table 1. Sampling data of the isolates used in this study

Species	Accession no.	GenBank	Source
Penicillium marneffei	CBS101038		Assam, India
	CBS555.90^T		Australia
	CBS388.87		Vietnam
	CBS122.89		Indonesia
	CBS440.88		USA
	SUMS0266	FJ009553	Bamboo rat spleen, Jiangxi, China
	SUMS0267	FJ009551	Bamboo rat spleen, Jiangxi, China
	SUMS0264	FJ009566	Bamboo rat kidney, Jiangxi, China
	SUMS0268	FJ009565	Bamboo rat spleen, Jiangxi, China
	SUMS0272	FJ009555	Bamboo rat lung, Jiangxi, China
	SUMS0344	FJ009554	Bamboo rat spleen, Shaoguan, China
	SUMS0345	FJ009559	Bamboo rat kidney, Shaoguan, China
	SUMS0346	FJ009560	Bamboo rat lung, Shaoguan, China
	SUMS0347	FJ009564	Bamboo rat liver, Fujian, China
	SUMS0348	FJ009563	Bamboo rat lung, Fujian, China
	SUMS0349	FJ009552	Bamboo rat liver, Shaoguan, China
	SUMS0350	FJ009557	Bamboo rat lung, Shaoguan, China
	SUMS0351	FJ009562	Bamboo rat lung, Shaoguan, China
	SUMS0352	FJ009558	Bamboo rat spleen, Shaoguan, China
	SUMS0353	FJ009561	Bamboo rat lung, Shaoguan, China
	SUMS0354	FJ009556	Bamboo rat lung, Shaoguan, China
	IFM47289^c	AB298957	Chiba University, Japan
	IFM47288	AB298956	Chiba University, Japan
	IFM47287	AB298955	Chiba University, Japan
	IFM47286	AB298954	Chiba University, Japan
	IFM47285	AB298953	Chiba University, Japan
	SUMS0152	AB353913	Human blood and bone marrow, Guangdong, China
	SUMS0112	AB353909	Human, Jiangxi, China
	SUMS0165	AB353908	Human blood, Guangdong, China
	SUMS0186	AB353917	Human face and blood, Guangdong, China
	SUMS0178	AB353916	Human blood, Guangdong, China
	SUMS0174	AB353915	Human neck, Guangdong, China
	SUMS0164	AB353914	Human face, Guangdong, China
	SUMS0096	AB353912	Human dialysate, Guangdong, China
	SUMS0050	AB353911	Animal, Guangxi, China
	SUMS0051	AB353910	Human, Guangxi, China
	SUMS0111	AB353919	Human, Guangdong, China

Table 1. Continued.

Species	Accession no.	GenBank	Source
	SUMS0187	AB353918	Human blood, Guangdong, China
	SUMS0107	AB353907	Human legs, Guangdong, China
	SUMS0047	AB353906	Human legs, Guangdong, China
Aspergillus fumigatus	SUMS0106	FJ011537	Human, Shanghai, China
	SUMS0317	FJ011543	
A. flavus	SUMS0060	FJ011539	Human sputum, Guangdong, China
	SUMS0062	FJ011545	Human lung, Guangdong, China
A. niger	SUMS0061	FJ011541	Human acoustic meatus, Guangdong, China
	SUMS0037	FJ011542	Human, Guangdong, China
A. terreus	SUMS0191	FJ011538	Human acoustic meatus, Guangdong, China
	SUMS0113	FJ011536	Human sputum, Guangdong, China
Penicillium griseofulvum	SUMS0392	FJ011548	Human face, Guangzhou, China
Paecilomyces variotii	SUMS0303	FJ011547	Human, Guangzhou, China
Penicillium janthinellum	IFM40620		IAM 7018
P. chrysogenum	IFM5338		MTU7003
P. purpurogenum	IFM40627		IAM7095
P. citrinum	IFM40616		IAM7003
P. duclauxii	CBS187.89		NRRL 2020
P. verruculosum	CBS101366		Soil, Hong Kong
P. minioluteum	CBS442.89		Soil, Lyngby, Denmark
P. crustosum	IFM47479		IFO31913 silk-worm foods
P. funiculosum	IFM57310-11		CBS235.94, ATCC11797 MD
P. pinohilum	IFM57309-L1		CBS631.66, IMI114933, ATCC36839
Talaromyces flavus	IFM42233		F-S-1
T. trachyspermus	IFM42251		M-2143
T. stipitatus	IFM42240		NHL 6092
T. thermophis	IFM52998		ATCC10518, CBS236.58, IMI48593
T. derxii	CBS413.89		Cultivated soil, Okayama Prefecture, Kurashiki City, Higashitomii, Japan
T. intermedius	CBS152.65		Alluvial pasture and swamp soil, Nottingham, Attenborough, UK
Blastomyces dermatitidis	IFM40753		From Jyunntenndo University
Coccidioides immitis	IFM45811		Patient, San Jose, AZ
Paracoccidioides brasiliensis	IFM41620		Patient, E. Burger
Sporathrix schenckii	SUMS0382	FJ011549	Human, Guangzhou, China
	SUMS0383	FJ011550	Human, Guangzhou, China
Fonsecaea pedrosoi	CBS272.37^T		Brazil
F. monophora	CBS269.37^T	AY857511	South America
Histoplasmo capsulatum	SUMS0035	AB353921	
H. duboisii	IFM5417		MTU 16024, TIMM0738, IP638
H. farciminosum	IFM41335		CDC B-22, L. Ajiello
Cryptococcus neoformans	SUMS0167	AB436650	Human cerebrospinal fluid, Guangzhou, China
	SUMS0042	AB436636	Human cerebrospinal fluid, Guangzhou, China
Candida albicans	ATCC90028	AB049119	Unknown
C. tropicalis	SUMS0125	FJ011533	Human blood, Guangzhou, China
C. parapsilosis	ATCC22019	AB105209	Unknown
C. krusei	ATCC6258	AB105208	Unknown
C. guilliermondii	ATCC6260	AF022717	Unknown
C. glabrata	ATCC2001	AF134719	Unknown
C. dubliniensis	SUMS0393	FJ011546	Human sputum, Guangzhou, China
Escherichia coli	ATCC25922	DQ360844	Shangdong, China

CBS, Centraalbureau voor Schimmelcultures, Baarn, the Netherlands; SUMS, Sun Yat-Sen University Medical Science, Guangzhou, China; IFM, Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, Chiba, Japan; ATCC, American type culture collection, Rockville, MD; ^{T}Type strain; IAM, Institute of Applied Microbiology, University of Tokyo, Tokyo, Japan; MTU, Department of Bacteriology, Faculty of Medicine, University of Tokyo, Tokyo, Japan; IFO, Institute for Fermentation, Osaka, Japan; IMI, CAB International Mycological Institute, Kew, UK; NHL, National Collection of Pathogenic Fungi, Mycological Reference Laboratory, Central Public Health Laboratory, London, UK; TIMM, Research Center for Medical Mycology, Teikyo University, Hachioji, Tokyo, Japan; IP, Unite de Mycologie, Institut Pasteur, Paris, France; CDC, Communicable Disease Centers, United States Public Health Services, Atlanta, GA; NRRL, Northern Regional Research Laboratory, Peoria, IL.

isolates were cultured on Sabouraud's glucose agar plates at 25 °C for 1 week; *Escherichia coli* was cultured in flasks shaken at 250 r.p.m. with Luria–Bertani at 37 °C overnight. About 0.5 g of mycelium or conidia, or precipitate of *E. coli*, respectively, were harvested for DNA extraction.

Twenty-three tissue samples from 23 patients (Zeng *et al.*, 2009) were selected. These included 12 samples from patients with proven penicilliosis marneffei, three from chromoblastomycosis, three from sporotrichosis, one from histoplasmosis, one from cryptococcosis, one from candidiasis, one from pulmonary aspergillosis and one from visually healthy human skin. Cases from human patients were confirmed by routine and molecular identification methods. *Penicillium marneffei* was also isolated from 10 of 11 bamboo rat tissue samples; one (bamboo rat liver) was used as a negative control. The time that elapsed after paraffin embedding of the tissue samples ranged between one day and 13 years. About 10-μg sectioned paraffin material was used for DNA extraction.

DNA extraction and quality test

Fungal DNA from pure culture was extracted using 6% InStaGeneTMMatrix (Bio-Rad, CA) as described previously (Xi *et al.*, 2009). Crude DNA of paraffin wax-embedded tissue was extracted from approximately 10-μg sections of paraffin wax-embedded tissue using the QIAamp® FFPE Tissue Kit (Qiagen, Hilden, Germany) according to Zeng *et al.* (2009). DNA concentrations were measured spectrophotometrically at 260 nm (Shimadzu Corp., Japan). DNA quality was confirmed by successful PCR amplification using universal fungal primers internal transcribed spacer (ITS)4 and ITS5 (Zeng *et al.*, 2009). PCR was performed as follows: 95 °C for 5 min; 35 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 1 min, with a final extension at 72 °C for 10 min. Amplicons were detected by electrophoresis (Bio-Rad) on a 2% agarose gel (NuSieve, Rockland, ME).

Design of LAMP primers

Four sets of 24 species-specific primers were designed based on the rRNA gene ITS region of *P. marneffei* SUMS0152 (AB353913) (Liu *et al.*, 2007; Xi *et al.*, 2007) using PRIMER-EXPLORER v4 software (http://primerexplorer.jp). A set of six species-specific LAMP primers was selected as follows: forward outer primer (F3): CCG AGC GTC ATT TCT GCC, reverse outer (B3): AGT TCA GCG GGT AAC TCC T, forward inner primer (FIP): TCG AGG ACC AGA CGG ACG TCT TTT TCA AGC ACG GCT TGT GTG, reverse inner (BIP): TAT GGG GCT CTG TCA CTC GCT CTT TTA CCT GAT CCG AGG TCA ACC, loop forward (LF): GTT GGT CAC CAC CAT ATT TAC CA and loop reverse (LB): TGC CTT TCG GGC AGG TC.

LAMP reaction

LAMP was performed in 25-μL reaction volumes containing 0.25 μM of F3 and B3 each, 1.0 μM of FIP and BIP each, 0.5 μM of LF and LB each, 1.0 mM dNTPs, 1 M betaine (Sigma), 20 mM Tris-HCl (pH 8.8), 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 4 mM $MgSO_4$, 0.1% Triton X-100 and 8 U of Bst DNA large fragment polymerase (New England Biolabs), with 2 μL of crude DNA extract as the template. The reaction mixture, except Bst DNA polymerase, was denatured at 95 °C for 5 min and cooled on ice, followed by the addition of 1 μL Bst polymerase and incubation at 65 °C in a water bath for 60 min and final heating at 85 °C for 2 min to terminate the reaction.

DNAs of 40 *P. marneffei* and 46 reference strains were used as templates to evaluate the specificity of the LAMP assay. DNA of strain SUMS0152 was used as a positive control; reaction mixtures without *P. marneffei* DNA, i.e. healthy human skin DNA, healthy bamboo rat DNA and DNAs from *Penicillium purpurogenum*, *Penicillium funiculosum* and other biverticillate penicillia taxonomically close to *P. marneffei* were used as negative controls.

A recombinant plasmid (pT-IT12) was constructed as a template for establishing the detection limit of the LAMP assay. The ITS region of *P. marneffei* (603 bp) was amplified from SUMS0152 genomic DNA using primers ITS4 and ITS5 and subcloned into the pGEM-T Easy vector (Promega) according to the manufacturer's instructions. Detection limits were evaluated using 10-fold serial dilutions of plasmid pT-IT12. The plasmid DNA (0.32 μg μL^{-1}, equivalent to 8.067×10^{10} copies μL^{-1}) was 10-fold serially diluted and 2 μL of each dilution was used as a template for the LAMP reaction. DNA of *P. marneffei* SUMS0152 was used as a positive control; the reaction mixture without DNA was used as a negative control. To evaluate the inhibition of nontarget DNA in the LAMP assay, 2 μL crude DNA extract each of *P. marneffei* was added to the LAMP-negative samples, and then tested by LAMP again.

Visualization

Amplified products were analyzed by electrophoresis on 1% agarose gels, stained with ethidium bromide and photographed. A 100-bp DNA ladder was used as the molecular weight standard. LAMP reaction products were made visible by the addition of 2.0 μL of 10-fold diluted SYBR Green I (Cambrex Bio Science, Wokingham, UK) to each reaction tube separately; the change in the color of the solution was observed directly by the naked eye or using a UV transilluminator.

Application of LAMP to paraffin wax-embedded tissues

Crude-extracted DNA of 2 μL each from 34 paraffin wax-embedded tissues' samples was used as a template for LAMP

assays. The amplified products were analyzed by the naked eye or by electrophoresis.

Results

LAMP assays using a set of six species-specific LAMP primers yielded positive results in all *P. marneffei* strains, but remained negative in all isolates used for reference, including related biverticillate penicillia (Table 1). Amplification was completed within 1 h isothermally at 65 °C in a water bath. The products of the LAMP reaction could be detected by electrophoresis on 1% agarose gels and showed ladder-like patterns (Fig. 1). The products could also be made visible to the naked eye directly in Eppendorf vials or under UV transillumination after adding SYBR Green I dye. Positive reactions showed bright green fluorescence, whereas negative reactions remained light orange (Fig. 2). The detection limit of *P. marneffei* DNA by the LAMP assay was found to be two copies by electrophoresis (Fig. 3). The visual sensitivity obtained after adding SYBR Green I correlated with the sensitivity established on agarose gel (Fig. 4).

All 12 proven *P. marneffei*-positive tissue samples and 10 samples of bamboo rat tissue tested positive, whereas samples of unaffected human skin and the remaining tissue samples affected by other fungi and tested for comparison yielded a negative response (Table 2). The correspondence between the LAMP assays and the cultural and molecular results of the same tissue samples proved to be 100%. In the inhibition test, it was found that all LAMP-negative samples became positive after the addition of 2 μL crude DNA extract of *P. marneffei*.

Discussion

LAMP is a powerful innovative gene amplification technique providing a simple and rapid tool for early detection and identification of microbial diseases. Most developments in molecular diagnostics published recently concerned improvements in PCR methodology on DNA extracted from pure cultures or from clinical specimens. This had led to changes in the primer design and reaction temperature (Boehme *et al.*, 2007; Inacio *et al.*, 2008) and to integration with hybridization and enzyme-linked immunosorbent assay techniques (Nagamine *et al.*, 2002; Lee *et al.*, 2009). In the present study, we further developed and evaluated the LAMP assay, exemplified by the detection and identification of *P. marneffei* in DNA from pure cultures as well as in paraffin wax-embedded tissues. Compared with any detection method applied thus far, the method is very fast, as it

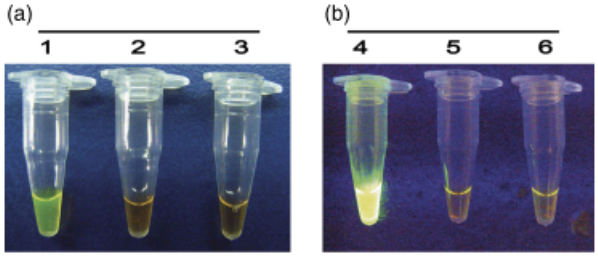

Fig. 2. Visual appearance of LAMP reactions from isolates after addition of SYBR Green I. (a) Positive reaction (tube 1), negative reaction (tube 2) and tube without DNA templates (tube 3). (b) Under UV transillumination, positive reaction (tube 4), negative reaction (tube 5) and tube without DNA templates (tube 6).

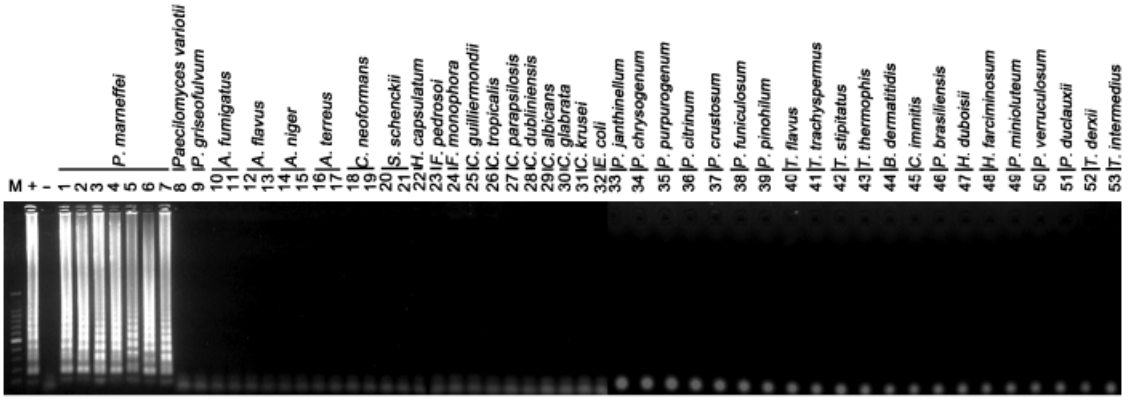

Fig. 1. Agarose gel electrophoresis of LAMP products from tested strains obtained using the primer set designed in this work. Left to right: lane 'M', 100-bp DNA marker; lane '+', SUMS0152 positive control; lane '−', negative control without DNA; lanes1–7, CBS101038, CBS555.90, IFM47287, SUMS0266, SUMS0347, SUMS0112, SUMS0051, respectively; lanes 8–53, SUMS0303, SUMS0392, SUMS0106, SUMS0317, SUMS0060, SUMS0062, SUMS0061, SUMS0037, SUMS0191, SUMS0113, SUMS0167, SUMS0042, SUMS0382, SUMS0383, SUMS0035, CBS272.37, CBS269.37, ATCC6260, SUMS0125, ATCC22019, SUMS0393, ATCC90028, ATCC2001, ATCC6258, ATCC25922, IFM40620, IFM5338, IFM40627, IFM40616, IFM47479, IFM57310-11, IFM57309-L1, IFM42233, IFM42251, IFM42240, IFM52998, IFM40753, IFM45811, IFM4162, IFM5417, IFM41335, CBS442.89, CBS101366, CBS187.89, CBS413.89, CBS152.65, respectively.

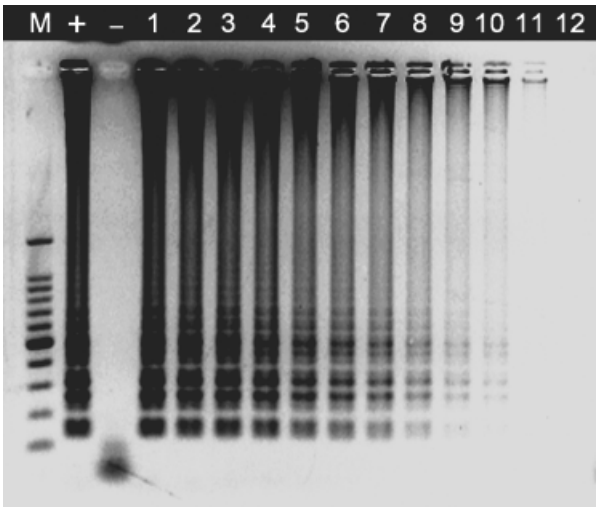

Fig. 3. Analytical sensitivity of LAMP for detection of the ITS1–5.8S–ITS2 rRNA gene. Left to right: Lane 'M', 100-bp DNA ladder; lane '+', SUMS0152 positive control; lane '–', negative control without DNA; lanes 1–12, 2×10^9, 2×10^8, 2×10^7, 2×10^6, 2×10^5, 2×10^4, 2×10^3, 2×10^2, 2×10^1, 2×10^0, 2×10^{-1} and 2×10^{-2} copies per tube, respectively.

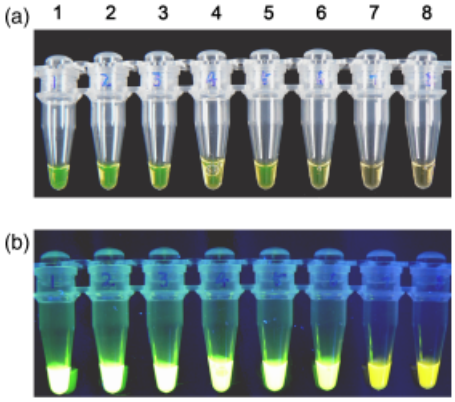

Fig. 4. Visual sensitivity of LAMP reactions using SYBR Green I. (a) Direct detection by the naked eye, (b) under UV transillumination. Tubes 1–7, 2×10^5, 2×10^4, 2×10^3, 2×10^2, 2×10^1, 2×10^0, 2×10^{-1} copies per tube, respectively; tube 8, negative control without DNA.

can be carried out within 1 h. It also does not require expensive laboratory equipment, because the method can be carried out isothermally at 65 °C in a water bath. Further, it is simple to use in a routine laboratory, as the results can be observed directly by the naked eye (Fig. 3). In addition, the detection limit is very low. With only two DNA copies, it has a higher sensitivity than the currently applied molecular methods, such as semi-nested PCR (10 pg) (Prariyachatigul *et al.*, 2003), PCR enzyme immunoassay (3.2 pg) (Lindsley *et al.*, 2001), PCR hybridization (0.1 pg) (Vanittanakom *et al.*, 1998) and nested PCR (0.07 pg) (Zeng *et al.*, 2009).

The results of *P. marneffei* detection by LAMP in 23 paraffin wax-embedded clinical samples and 11 bamboo rat tissues were also highly specific. The etiologic agents of the 23 clinical samples were verified previously using culture and sequencing data. Twelve samples were histopathologically positive; all molecular identifications matched with the clinical diagnoses. Samples from penicilliosis and from the natural bamboo rat host were positive with LAMP, whereas all others, including healthy human skin, proved to be negative. Test results were not inhibited by nontarget DNA. This makes the LAMP technique highly promising for evaluation and application in problematic clinical samples such as blood, urine and sputum.

Table 2. Biopsy specimens used in this study and the results of different detection methods

		Detection method		
Sample no.	Source	Culture and ITS sequencing	PAS	LAMP
Paraffin wax-embedded tissues				
1	Human, skin	*Penicillium marneffei*	+	+
2	Human, skin	*P. marneffei*	+	+
3	Human, skin	*P. marneffei*	–	+
4	Human, skin	*P. marneffei*	–	+
5	Human, lung	*P. marneffei*	+	+
6	Human, skin	*P. marneffei*	+	+
7	Human, lymph node	*P. marneffei*	+	+
8	Human, skin	*P. marneffei*	+	+
9	Human, skin	*P. marneffei*	+	+
10	Human, skin	*P. marneffei*	+	+
11	Human, skin	*P. marneffei*	+	+
12	Human, lung	*P. marneffei*	+	+
13	Human, skin	*Sporathrix schenckii*	–	–
14	Human, skin	*S. schenckii*	–	–
15	Human, face	*S. schenckii*	–	–
16	Human, lung	*Aspergillus* sp.	–	–
17	Human, leg	*Fonsecaea pedrosoi*	–	–
18	Human, leg	*F. pedrosoi*	–	–
19	Human, abdominal skin	*F. pedrosoi*	–	–
20	Human, face	*Cryptococcus neoformans*	–	–
21	Human, leg	*Histoplasma capsulatum*	+	–
22	Human, skin	*Candida albicans* *Candida parapsilosis*	+	–
23	Human normal skin	–	–	–
Bamboo rat tissues				
1	Bamboo rat, kidney	*P. marneffei*	–	+
2	Bamboo rat, spleen	*P. marneffei*	–	+
3	Bamboo rat, lung	*P. marneffei*	–	+
4	Bamboo rat, liver	*P. marneffei*	–	+
5	Bamboo rat, lung	*P. marneffei*	–	+
6	Bamboo rat, liver	*P. marneffei*	–	+
7	Bamboo rat, lung	*P. marneffei*	–	+
8	Bamboo rat, spleen	*P. marneffei*	–	+
9	Bamboo rat, lung	*P. marneffei*	–	+
10	Bamboo rat, liver	*P. marneffei*	–	+
11	Bamboo rat, lung	*P. marneffei*	–	+
12	Bamboo rat, liver	–	–	–

In this study, we have proved with the example of *P. marneffei* that LAMP is a very efficient method for the quick and sensitive identification of fungal pathogens and opportunists. The method can be applied not only to cultures but also to a variety of clinical samples. This can be of great significance to organisms that cause invasive or disseminated infections that are difficult to cultivate from such samples, such as the zygomycete species. A further application may be for detection without isolation of the fungi in the environment.

In summary, in the current study, we proved that the LAMP technique enables specific detection of *P. marneffei* and excludes related biverticillate penicillia and *Talaromyces* teleomorphs. Similar results were obtained in *Paracoccidioides* (Endo *et al.*, 2004), *Candida* (Inacio *et al.*, 2008) and *Ochroconis* (Ohori *et al.*, 2006). However, in *Fonsecaea*, identification was possible only at the generic level (Najafzadeh, 2009). An explanation for this phenomenon may be found in the fact that *Penicillium* species are relatively distant from each other, with ITS barcoding gaps well over 1%, whereas in *Fonsecaea* ITS, interspecific differences are a few bases only, species delimitations being based on multilocus analyses.

Acknowledgements

We thank Prof. Yokoyama (Center for Pathogenic Fungi and Microbial Toxicoses Chiba University, Chiba, Japan) for providing the reference strains taxonomically close to *P. marneffei* included in this paper. This study was supported partly by a grant (30770121/2007) from the National Natural Science Foundation of China.

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