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CASE REPORT/CAS CLINIQUE

Novel intein-containing DNA specific primers for rapid identification of *Candida glabrata* using Real-Time PCR assays

Nouvelles amorces spécifiques d'ADN contenant une intéine pour l'identification rapide de Candida glabrata *en utilisant des tests PCR en temps réel*

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KEYWORDS
C. glabrata;
Intein;
Real-Time PCR;
Rapid detection

Summary *Candida glabrata* is an opportunistic human pathogen known to cause systemic and vaginal candidiasis. Rapid detection of *Candida glabrata* is indispensable for appropriate selection of antifungal drugs for chemotherapy. The study describes a unique intein-containing DNA fragment for specific detection of *C. glabrata*. The designed oligonucleotides detected *C. glabrata* (C_t mean: 24.75 ± 1.1 and T_m: 70.08 ± 0.23 °C) in Real-Time PCR assays. The fluorescent signals were negative when the primers were tested for cross-species and cross-genera amplifications. In conclusion, our study recommends a novel primer set for developing a quick identification system which does not require laborious and time-consuming experimentations.

MOTS CLÉS
C. glabrata ;
Intéine ;
PCR en temps réel ;
Détection rapide

Résumé *Candida glabrata* est un agent pathogène humain opportuniste connu pour causer des candidoses systémiques et vaginales. La détection rapide de *Candida glabrata* est indispensable pour la sélection appropriée des médicaments antifongiques pour la chimiothérapie. Cette étude décrit un fragment unique d'ADN contenant une intéine pour la détection spécifique de *C. glabrata*. Les oligonucléotides conçus ont détecté *C. glabrata* (C_t moyenne : 24,75 ± 1,1 et T_m : 70,08 ± 0,23 °C) dans des tests PCR en temps réel. Les signaux fluorescents ont été négatifs lorsque les amorces ont été testées pour des amplifications inter-espèces et inter-genres. En conclusion, notre étude recommande un nouveau jeux d'amorces pour

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l'élaboration d'un système d'identification rapide qui ne nécessite pas d'expérimentations laborieuses et chronophages.

Introduction

Candida glabrata is one of the leading non-albican yeast species often allied with systemic and vulvovaginal candidiasis [2,9]. For decades, *C. glabrata* was considered as a non-pathogen, but recent reports suggest that the antibiotic resistance mechanisms and evolutionary pressure has led to the emergence of *C. glabrata* variants which are highly pathogenic and drug-resistant [10]. Moreover, therapeutic strategies, like of immunosuppressive drug therapy and antimycotic therapies have drastically increased *C. glabrata* infections [13]. The vast majority of *C. glabrata* are less susceptible to azoles antifungal drugs [3]. Erroneous diagnosis and false-positive interpretations have rigorous impact on drug selection for antifungal chemotheraphy. Detection and discrimination of *Candida* spp. causing candidiasis is still a grey area. Real-Time PCR assays with non-specific fluorescent dye SYBR® Green is a pre-eminent choice for molecular detection of fungal pathogens [11,19], but again, it requires precise selection of the target sequences. Internal transcribed spacer (ITS) regions are molecular targets for detection of *Candida* spp. Sequencing of ribosomal ITS and intragenic spacer (IGS) regions are the standard method adopted by mycologist to identify the species [6,7]. Several other functional genes (lanosterol-alpha demethylase and chitin synthase) and hypothetical proteins (CBS138 hypothetical protein) were targeted for detection of *C. glabrata* [1,4,12]. These genes are commonly dispersed within the microbial community and may result in cross reactivity. Moreover, the amplification relies on the primer binding sequences in yeast genome, which may tend to change during the course of evolution.

Hence, the choice of unique DNA fragments for detection of *C. glabrata* is still a challenge. Our study highlights on intein-encoding DNA fragment as an alternate and promising choice for designing species-specific primers for detection of *C. glabrata*. Inteins are parasitic genetic elements located on highly conserved genes. Hence, inteins are promising

Table 1 Fungal strains used in the study.
Souches fongiques utilisées dans l'étude.

Strain No.	Origin	Species	Source	Strain designation	C_t value (mean ± SD)	Presence/absence (±) analysis
3019	Iowa, USA	*C. glabrata*	MTCC	NCCLS 84	24.97 ± 0.01414	+
3981	Assam, India	*C. glabrata*	MTCC	AB—Y7	24.96 ± 0.00	+
3982	Pokhra, Nepal	*C. glabrata*	MTCC	AB—Y8	24.45 ± 0.70711	+
3983	Assam, India	*C. glabrata*	MTCC	AB—Y9	23.95 ± 0.00	+
6507	Madurai, India	*C. glabrata*	MTCC	SPG—1	23.46 ± 0.72125	+
3985	Arunachal Pradesh, India	*C. glabrata*	MTCC	AB—Y11	24.47 ± 0.72125	+
3988	Assam, India	*C. glabrata*	MTCC	AB—Y14	23.47 ± 0.70711	+
3814	Sikkim, India	*C. glabrata*	MTCC	MS—YC5	24.955 ± 1.42128	+
3984	Tezpur, India	*C. glabrata*	MTCC	AB—Y10	25.455 ± 0.71418	+
3986	Tezpur, India	*C. glabrata*	MTCC	AB—Y12	23.955 ± 1.40714	+
3987	Tezpur, India	*C. glabrata*	MTCC	AB—Y13	25.975 ± 0.00707	+
2001	CBS	*C. glabrata*	ATCC	CBS 138	25.965 ± 0.00707	+
3017	Iowa, USA	*C. albicans*	MTCC	NCCLS 11	NFS	—
3018	ZA McGee, Canada	*C. albicans*	MTCC	ATCC 24433 Wasson	NFS	—
184	Pune, India	*C. tropicalis*	MTCC	3118	NFS	—
230	—	*C. tropicalis*	MTCC	997	NFS	—
9215	—	*C. krusei*	MTCC	—	NFS	—
2512	Roorkee, India	*C. parapsilosis*	MTCC	PD—10	NFS	—
9687	Goa, India	*A. niger*	MTCC	GUFCC 5443	NFS	—
10180	West Bengal, India	*A. niger*	MTCC	—	NFS	—
1884	Madhya Pradesh, India	*A. flavus*	MTCC	PD—2	NFS	—
2797	USA	*A. parasiticus*	MTCC	SRRC1008	NFS	—
6569	Arunachal Pradesh, India	*F. oxysporum*	MTCC	—	NFS	—
2671	Solan, India	*F. solani*	MTCC	I	NFS	—
251	NCYC, UK	*S. cerevisiae*	MTCC	913	NFS	—

ATCC: American type culture collection; MTCC: Microbial type culture collection; NFS: No fluorescent signal; Ct: Cycle threshold.