ABSTRACT BOOK

2nd International Conference on Advance Molecular Bioscience and Biomedical Engineering (ICAMBBE) 2015

Institute Biosains
University of Brawijaya
Malang, East Java, Indonesia

ABSTRACT BOOK

2nd International Conference on Advance Molecular Bioscience and Biomedical Engineering (ICAMBBE) 2015

Institute Biosains Laboratory
Brawijaya University
Malang, East Java, Indonesia
August, 13th – 14th 2015

Atria Hotel and Convention
Jl. Letjen S Parman 87-89 Malang
East Java-Indonesia

MM-03

Identification of yeast candida genus using multiplex-PCR for diagnostic purpose application

Kemas Yakub Rahardiyanto[1], Susilawati[1]*, Hermansyah[2]

[1]*Faculty of Medicine, Sriwijaya University, Palembang*
[2]*Chemistry Department, Faculty of Mathematics and Natural Sciences, South Sumatera*
Email: susilwt78@yahoo.com

The objective of this study was to identify yeast Candida species using multiplex-Polymerase Chain Reaction (PCR) as a potential method in detecting candidiasis causes. In this research, two primer pairs, universal primer ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') - ITS2 (5'-GC TGCGTTCTTCATCGATGC-3') and specific primer CA3 (5'-GGTTTGCTTGAAAGACGGTAG-3') and CA4 (5'-AGTTTGAAGATATACGTGGTAG-3'). Based upon of the length of DNA fragments resulted from PCR can be used to identify Candida species. *C. glabrata* (482 bp-483 bp atau 462 bp-463 bp), *C. guillermondii* (248 bp atau 228 bp), *C. parapsilosis* (229 bp atau 209 bp), *C. tropicalis* (218 bp atau 199 bp), *C. albican* (218 bp-219 bp atau 198 bp-199 bp dan 110 bp), *C. crusei* (182 bp atau 166 bp), *C. lusitaniae* (148 bp atau 128 bp), *C. dubliniensis* (198 bp), *C. stellatoideae* (190 bp). These results suggested that the Multiplex-PCR method could be apply as alternative diagnostic test for Candidiasis causes.

Keywords: multiplex-PCR, ITS1-ITS2, CA3 and CA4, *Candida sp.*

Identification of Yeast Candida Genus Using Multiplex-PCR for Diagnostic Purpose Application

Kemas Yakub Rahardiyanto[1], Susilawati[1)*], Theodorus[1], Muhaimin Ramdja[1], Hermansyah[2]

[1]Faculty of Medicine, Sriwijaya University, Jalan Dr. Moh.Ali, Komplek RSMH Palembang 30126.
[2]Chemistry Department, Faculty of Mathematics and Natural Sciences, Inderalaya, Ogan Ilir, South Sumatera 30662.
*)Correspondencing Author : susilwt78@yahoo.com

ABSTRACT

The objective of this study was to identify yeast Candida species using multiplex-Polymerase Chain Reaction (PCR) as a potential method in detecting candidiasis causes. In this research, two primer pairs, universal primer ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') - ITS2 (5'- GC TGCGTTCTTCATCGATGC-3') and specific primer CA3 (5'- GGTTTGCTTGAAAGACGGT AG-3') and CA4 (5'- AGTTTGAAGATATACGTGGTAG-3'). Based upon of the length of DNA fragments resulted from PCR can be used to identify Candida species. *C. glabrata* (482 bp-483 bp atau 462 bp-463 bp), *C. guillermondii* (248 bp atau 228 bp), *C. parapsilosis* (229 bp atau 209 bp), *C. tropicalis* (218 bp atau 199 bp), *C. albican* (218 bp-219 bp atau 198 bp-199 bp dan 110 bp), *C. crusei* (182 bp atau 166 bp), *C. lusitaniae* (148 bp atau 128 bp), *C. dubliniensis* (198 bp), *C. stellatoideae* (190 bp). These results suggested that the Multiplex-PCR method could be apply as alternative diagnostic test for Candidiasis causes.

Keywords: Multiplex-PCR, ITS1-ITS2, CA3 and CA4, *Candida sp*

Introduction

The implication of Candida sp inspection in reproductive canal has to pay more attention due to harming the women like getting Leukorea, itching which caused abrasions and uncomfortable sexual intercourse. Candidiasis vaginalis also can cause candida chorioamnionitis that led to abortus or an infant born with congenital candidiasis, and more than 75 % woman had endured at least one episode candidiasis vaginalis during his lifetime, and around half of them have vaginalis candidiasis recurring (Omar, 2001). The cause of candidiasis vaginalis at several locations in Jakarta is *candida albicans* or 62.3 % and non-candida albicans 30.4 % , consist of:

candida glabrata 18.8 % , candida tropicalis 8.7 % , candida parapsilosis 2.9 % and a mixed infection of total of 7.3 % (Haryani *et al* . , 2002) . `

Success of treatment depends on the sensitivity of Candida species against anti-fungal. Species level identification is needed early in the case of candidiasis caused by Candida sp which is resistant to the azole so that alternative therapies can be immediately given. Conventional methods (Fermentation-Assimilation) as the Gold Standard has a high sensitivity and specificity but require a long examination time (7-21 days) and troubles in terms of the provision of chemicals as well as the costs are quite high (Wahyuningsih *et al.* , 2012). At this time many molecular test that has been developed for detecting Candida species due to its perceived need faster, more sensitiv and more specific. One approach is molecular test Multiplex-PCR which is the development, modification, and a variation of the method of analysis of the PCR in which two or more simultaneous diamplifikasi locus in the same reaction making it possible to have a sensitivity.

Materials and Methods

Isolates and Medium

Candida spp isolates were obtained from the patients who detected candidiasis vaginalis clinic in Policlinic of Graha Sriwijaya collected during the period May 2005. Isolates were grown on Sabouraud medium (20 g/L Dextrose, 10 g/L Peptone. 17 g/L bacto agar and 0.25 g/L chloramphenicol.

DNA Extraction

DNA genome Candida spp isolates was extracted as described in da Silva *et al* (2012) with some modifications. Cells were cultured in 10 mL Sabouraud medium at 30°C for overnight, and they were harvested after centrifugation at 5000xg. An aliquot of 1000 µL of cell suspension containing 10^7 cells/mL of isolates was transferred to 1.5 mL eppedorf tube and incubated at boiling water bath for 5 min. The suspension containing DNA was vigorously homogenized by vortex for 15 s and the tube was frozen on ice. Boiling and vortex treatments were three times repetition.

Multiplex-PCR

Multiplex–PCR method was performed using JumpStart REDTaq ReadyMix (Sigma Aladrich) following manufacturer's procedure. PCR amplifications were carried out in 10 μL reaction mix. JumpStart REDTaq ReadyMix consisted of 20 mM Tris-HCl, pH 8.3, 100 mM KCl, 4 mM $MgCl_2$, 0.002 % gelatin, 0.4 mM each dNTP (dATP, dCTP, dGTP, dTTP), inert dye, stabilizers, 0.03 unit/mL Taq DNA polymerase, and JumpStart Taq antibody. Primer pairs consisted of ITS1: (5'-TCCGTAGGTGAACCTGCGG-3') and ITS2: (5'- GCTGCGTTCTTC ATCGATGC-3'); CA3: (5'- GGTTTGCTTGAAAGACGGTAG-3') and CA4: (5'-AGTTTGAAGATATACGTGGTAG-3') to amplify the conserved region of 18S rDNA (Tarini *et al*, 2010). Amplification condition of PCR as follows : an initial denaturation step at 95°C for 15 sec, followed by 4o cycles comprising a denaturation at 94°C for 30 sec, an annealing step at 60°C for 1 min, and extension step at 72°C for 45 sec, followed by a final extension at 72°C for 5 min.

Electrophoresis

A 5.0 μL of PCR-amplified product and the 100-bp DNA Ladder were loaded onto 2% agarose electrophoresis gels (Sigma chemical, USA). The run was performed with 1 % Tris-acetate-EDTA (TAE) buffer, at 100 V/cm for 30 min. The gels were photographed using Gel Doc 1000 (Biorad, USA) and visualized using software quantity one (Biorad, USA).

Results and Discussions

Fragment size resulted from PCR-amplification using two primer pairs, universal primer ITS1-ITS2 and specific primer CA3-CA4 were analyzed using previous report (Liguori *et al*, 2006) as follows : *C. glabrata* (482 bp-483 bp atau 462 bp-463 bp), *C. guillermondii* (248 bp atau 228 bp), *C. parapsilosis* (229 bp atau 209 bp), *C. tropicalis* (218 bp atau 199 bp), *C. albican* (218 bp-219 bp atau 198 bp-199 bp dan 110 bp), *C. crusei* (182 bp atau 166 bp), *C. lusitaniae* (148 bp atau 128 bp), *C. dubliniensis* (198 bp), *C. stellatoideae* (190 bp).

Although 68 samples were collected from the patients of "Graha Sriwijaya" policlinic however only 30 samples were identified using this multiplex-PCR. A total of 30 isolates samples were analyzed as presented in Figure 1. The most frequently isolated species was *C.crusei* (14 isolates, 46.7%), followed *C.crusei* + *C.glabrata* (5 isolates, 16.7%), C.tropicalis (5 isolates, 16.7%), *C.albicans* (1 isolate, 3.3%), *C.stellatoideae* (3 isolates, 10%) and

C.stellatoideae + *C.glabrata* (2 isolates, 6.7%) (Table 1). Length of the PCR product resulted from multiplex PCR using ITS1-ITS2 and CA3-CA4 primer pairs were clearly could be visualized by agarose gel electrophoresis (Fig 1). Isolates were identified as *C. glabrata, C.tropicalis, C.albicans,* and *C.crusei* since they produced fragment length 483 bp; 218 bp; 218 bp and 110 bp; 182 bp, respectively. By multiplex PCR method, *C.albicans* could be identified more accurate and specific using specific primer CA3 and CA4, therefore we can easier identify *C.albicans* and *C. Stellatoideae*. C.

Table 1. Identification of *Candida spp* by multiplex PCR using ITS1-ITS2 and CA3-CA4 primer pairs

No. Sampel	**Multiplex-PCR**
2	*C. tropicalis*
4	*C. tropicalis*
5	*C. stellatoideae*
6	*C. crusei, C. glabrata*
7	*C. stellatoideae*
12	*C. crusei*
13	*C. crusei*
18	*C. crusei*
19	*C. crusei, C. glabrata*
20	*C. stellatoideae, C. glabrata*
25	*C. crusei*
31	*C. crusei*
32	*C. crusei*
35	*C. crusei*
37	*C. tropicalis*
39	*C. crusei*
41	*C. tropicalis*
44	*C. albican*
53	*C. crusei, C. glabrata*
54	*C. crusei*
58	*C. crusei*
60	*C. crusei*
61	*C. stellatoideae*
63	*C. crusei, C. glabrata*
65	*C. crusei*
74	*C. crusei*
75	*C. crusei*
77	*C. tropicalis*
79	*C. crusei, C. glabrata*
80	*C. stellatoideae, C. Glabrata*
Jumlah	30

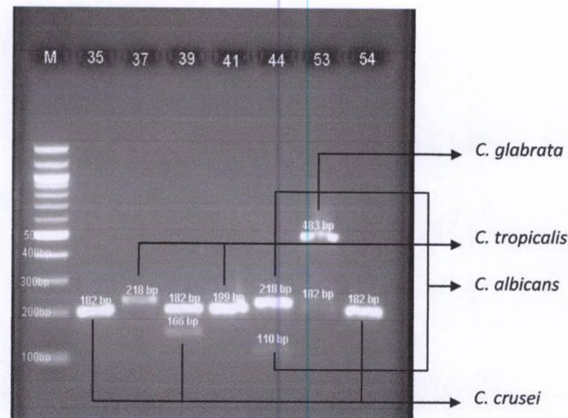

Figure 6. Visualization of fragment length of Candida species resulted from multiplex PCR method using ITS1-ITS2 and CA3-CA4 primer pairs.

By multiplex-PCR method, identification of Candida species is much easier since identification can be conducted in less time, for example 7 Candida species can be identified all in once time (Makene, 2014.)

Conclussion.

1. The most frequently isolated species was *C.crusei* (14 isolates, 46.7%), followed *C.crusei* + *C.glabrata* (5 isolates, 16.7%), C.tropicalis (5 isolates, 16.7%), *C.albicans* (1 isolate, 3.3%), *C.stellatoideae* (3 isolates, 10%) and *C.stellatoideae* + *C.glabrata* (2 isolates, 6.7%).
2. Multiplex-PCR method can be alternative method to identify Candida species.

Acknowledgment

The authors would like to thank to DIKTI and UNSRI for financial support of this Hibah bersaing grant 2015 research project.

References :

da Silva, G.A., T. L. Bernardi, P. D. C. Schaker, M. Menegotto, & P. Valente. 2012. Rapid Yeast DNA Extraction By Boiling and Freeze-Thawing Without Using Chemical Reagent and DNA Purification. An International Journal. 55(2): 319-327.

Haryani, M., K. S. Urip, & S. M. Wasitaatmadja. 2002. Vulvovaginal Candidosis Caused by *Candida Non-albicans,* Proportion and Clinical Characteristics in the Cipto Mangunkusumo National General Hospital, Jakarta. Medical Journal 12:142-146.

Liguori G., A. Lucariello, G. Colella, A. De Luca, & P. Marinelli. 2007. Rapid Identification of Candida Species in Oral Solution by PCR. J Clin Pathol. 60:1035-1039.

Makene, V. Anacletus. 2014. Identification of Non-Albicans Yeast Associated with Vulvovaginal Candidiasis in Tanzania Using a Combination of Multiplex-PCR and DNA sequence Divergence of the 26s LSU rDNA. Scholars Academic Journal of Biosciences (SAJB). 2(2):124-131.

Omar, A. A. 2001. Gram Stain Versus Culture in the Diagnosis of Vulvovaginal Candidiasis. Eastern Mediterranean Health Journal 7(6):925-932.

Tarini, Ni Made A., M. H. Wahid, F. Ibrahim, A. Yasmon, & S. Djauzi. 2010. Development of Multiplex-PCR Assar for Rapid Detection of *Candida spp.* Med J Indones 19:83-87.

Wahyuningsih, R., S. M. Eljannah, & Mulyati. 2012. Identifikasi *Candida* spp. Dengan Medium Kromogenik. J Indon Med Assoc 62(3).