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Molecular characterization of Mycovirus in the dermatophyte and non-dermatophyte fungi

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ABSTRACT: Mycoviruses are widespread in all major fungal groups and most of these cause little or no obvious symptoms in their fungal hosts. Mycoviruses particles were recorded since 1970s period. Many fungi like filamentous fungi like *Fusarium* spp. and mushroom *Lentinula edodes* infected by many types of viral particles but they were not regarded as causal agents for mushroom diseases. In this study, the dsRNA genome of a mycovirus recently found in dermatophyte and non-dermatophyte fungi and their molecular structure was characterized. Genomic DNA of 180 isolates of *Candida albicans* and *Trichophytone rubrum* was extracted and electrophoresis through agarose gel. The results showed that many types of viral genomic DNA were detected, The Partitiviridea and Reoviridea genome of were obtained. In conclusion we observed that many filamentous fungi carried viral particles in their culture without any plaques as a lysogenic form of mycophage.

KEYWORDS: Mycovirus, Dermatophyte, Non-dermatophytes, *Candida albicans*.

INTRODUCTION

Since 1970s periods many types of mycoviruses were isolated and recorded ,some of these viral particles infect the cultivated mushrooms such as Lentinula edodes were extensively studied in Japan(Ushiyama etal., 1973; Ushiyama et al.,1977 ; Mori et al., 1978).more one of tools were used for mycovirois detection like electron microscopy(Mori et al., 1978).some of these mycovirus was associated with healthy fruiting bodies (Mori et al., 1978;Fletcher et al.,2008). In the USA, dsRNAs have also been observed in shiitake strains, but these appeared to be latent (Pearson et al.,2009; Martin et al., 2011). However, abnormal symptoms are occasionally observed in plate cultures, such as plaque appearance on cultures texture and the growth of white or fluffy mycelia on the surface of substrate, inadequate or imperfect substrate browning (Ohta et al., 2008). Furthermore, a novel bipartite dsRNA mycovirus phylogenetically distantly related to Totiviruses and Chrysoviruses was reported from the white root rot fungus *Rosellinia necatrix* (Magae and Sunagawa 2010). More recently, several monopartite dsRNA viruses with evolutionary links between Totiviruses and partitiviruses were isolated from plants (Rytter et al., 1991; Ohta et al., 2008 ; Wu et al.,2012). dsRNA was found in two isolates; one showed imperfect browning and the other was asymptomatic. Agarose gel analysis showed that the isolate with imperfect browning contained several dsRNAs, but the asymptomatic isolate contained only a single dsRNA. As far as we know, no previous studies were performed in Iraq and neighboring regional countries which deal with mycoviruses.

MATERIEL AND METHODS

FUNGAL STRAIN AND CULTURE CONDITIONS

A total of 150 Clinical samples were obtained from out patients in Babylon province, middle of Iraq. Isolates were routinely cultured on SDA at 30- 35°C and stored at 4°C in SDA tube slants as subcultures.

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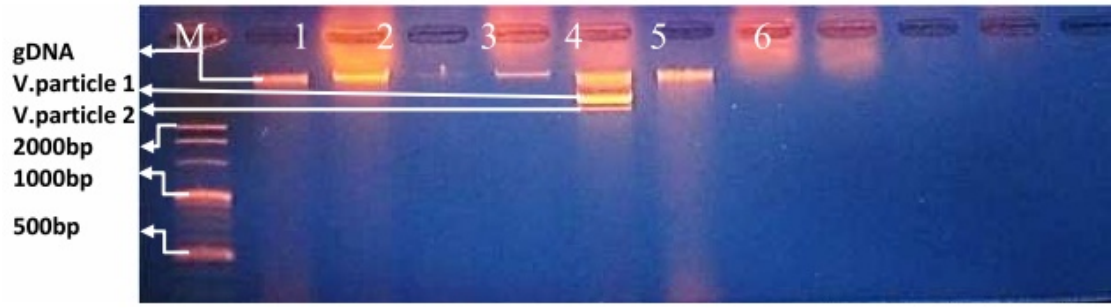
EXTRACTION OF GENOMIC DNA

A total of 180 fungal isolates were subjected to DNA extraction DNA of *Candida* isolates were extracted by pickup loop full of single colony , and tiny portions of mycelia of filamentous fungi were harvested from grown fungi on SDA at 30°C for 2-10 day, and then the mycelium was suspended into 300 µL of lysis buffer (10mM Tris, 1mM EDTA (pH8),1%SDS, 100mM NaCl)and heated at 85C for ten min. mixed with 300 µL phenol-chloroform (1:1) shaken for 5 min. and centrifuged at 5000 rpm, the supernatant transferred to new tube and added equal volume of chloroform, mixed ,centrifuged and the supernatant was transferred to new tube , 500 µl of isopropanol was mixed with supernatant and centrifuged at 12000 rpm for 7 min. the DNA pellet washed with 500 µl of ethanol alcohol and centrifuged at 7000 rpm for 3 min ,dry DNA pellet was re-suspended in 75 µl of TE buffer and stored at – 20^{o}C until use (Ramage et al.,2001) . were fractionated on 1.0% agarose gel and stained with ethidium bromide.

RESULTS AND DISCUSSION

All 180 clinical isolates were screened for the presence of putative dsRNA viruses, which were detectable as bright and distinct dsRNA bands with gel electrophoresis. two different dsRNA patterns were detected ranging from 1.0–2.2 kb. (Figure 1) Since genomes of Partitiviridea and Reoviridea were obtained have been observed in many fungal species and can be divided in 2 – 4 segments, our result coincidence with the results that obtained by Sabanadzovic et al.,2009; Spear et al.,2010. The phenotypic differences between infected and non-infected *T.rubrum* isolates were not observed.



Figure(1): Profile gDNA and nucleic acid of viruses, lanes M=Molecular marker 100bp for each step, lane 4 =2 bands of viral particles (large and medium viral bands).

The figure (1) displayed in gel plate two bands of viral like particle((large and medium bands of VLP) from dermatophtes in lane 4 =2 viral like particles isolated from *Trichophyton* dermatophytes fungi. Our result exhibited that most of *T.rubrubum* as pathogenic dermatophyte ,was the most common fungi associated with viral particles, the cultures of fungi not revealed any distractive symptom in culture plates . Agarose gel electrophoresis of dsRNA isolated from mycelial extracts of strains *T. rubrubum*. In our search it has been observed that largest L-dsRNA was generally migrated slightly slower than the M-dsRNA(Kozlakidis et al.,2009). The molecular weight of viral particles ranged from 1-2 kp in length our coincidence with Preisig et al., 1998 (Figure 1). Unfortunately no previous literature review available in Iraq and neighboring countries to compared and discussed this results with them . Our conclusion conducted that mycovirus to be correlated with human pathogenic fungi like *T. rubrubum*

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